FACULTY OF HEALTH AND MEDICAL SCIENCES
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Variations in plasma concentration in patients with non-small cell lung cancer on fixed-dose erlotinib

Department of Drug Design and Pharmacology

Master´s Thesis
by Khanda Amin

Study number: nbw867

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Preface

This Thesis was carried out as the final part in obtaining the Master of Science degree in Pharmacy at the University of Copenhagen.

The experimental work of this study was performed from January to April 2013 and took place at the Department of Pharmacy and Analytical Biosciences at the Faculty of Health and Medical Sciences, University of Copenhagen. This thesis is credited to 30 ECTS points.

I would like to thank my supervisor Professor Per Hartvig Honoré for his guidance and discussions throughout this project and for the opportunity to perform this interesting study.

I also want to thank Professor Steen Honoré Hansen and the laboratory technicians on the 6th floor at Department of Pharmacy and Analytical Biosciences for their assistance, collaboration and practical help in the laboratory.

Further thanks go to the Chief Physician Dr. Anders Mellemgaard at the Oncology department from Herlev Hospital for his assistance and help with applications which were sent to research ethics committee and to Danish Health and Midicines Authority.
Furthermore I thank the research nurse Rasmus Skøtt for collecting some patient data and blood samples.

Khanda Amin

Copenhagen, May 2013

Abstract

Introduction

Erlotinib is a tyrosine kinase inhibitor used as second line treatment in non-small cell lung cancer. The pharmacokinetics of erlotinib has high interindividual variation and hence variable clinical effect due formation by the Cyp P450 system subtype enzymes of a metabolite with pharmacological activity, influence of food on bioavailability and low clearance causing higher risk in liver impairment. There is only one dose recommended daily is hampering dose individualization.

Aim

The aim of this study is to evaluate and characterize the variations in plasma concentration of erlotinib and its possible formed main metabolite in order to estimate the person to person variability among patients with non-small cell lung cancer on fixed-dose erlotinib.

Method

13 patients received an oral, fixed daily dose of 150 mg of erlotinib and 4 patients received 100 mg daily for treatment of non small cell lung cancer. Plasma concentrations of erlotinib and its main active 4-hydroxyy-metabolite, OSI-420 were assessed.The analysis was performed by High Performance Liguid Chromatography HPLC with UV detection.

Result

The plasma concentrations of erlotinib were all in the range 0.76 to 3.63 mg/L with the lowest concentration measured in a patient taking the lower dose. Otherwise the plasma concentrations patients with the lower erlotinib daily dose of 100mg did not seem differ from those of the others. There was only one smoker in the group and his plasma concentration did not differ from other patients in the study. The eldery patients over 70 years of age did not show higher concentrations of erlotinib. The main metabolite was formed in significant amount and the ratio in the steady state situation ranged 0.076 to 0.42. The majority of patients were in the low range with 3 outliers with a ratio of about twice the others.

Conclusion

A bioanalytical method was developed and validated for this cross-sectional study on the interpatient variability of erlotinib pharmacokinetics, the variation in formation of a tentative active metabolite and steady state concentration reached for a good clinical effect. All patients seemed to have a plasma concentration above minimum effective level and all also had a significant fraction of 4-hydroxy metabolite formed in significant amount.

Abstrakt på dansk

Introduktion

Erlotinib er en tyrosin kinase hæmmer, den anvendes mod ikke småcellet lungekræft som anden linies behandling. Farmakokinetisk har erlotinib en høj interindividuel variation og dermed varierende klinisk effekt på grund af dannelse af en farmakologisk aktiv metabolt via Cyp P450 enzym subtyper. Fødevarer har indflydelse på biotilgængeligheden samt lav clearance medfører højere risiko for nedsat leverfunktion. Der er kun en anbefalet daglig dosis hvilket har forhindret dosisindividualisering.

Formål

Formålet med denne undersøgelse er at vurdere og karakterisere variationer af erlotinibs plasmakoncentration samt det mulige dannede hoved metabolit for at estimere person- person variationen blandt patienterne med ikke-småcellet lungekræft som er på fast dosis erlotinib.

Metode

13 deltagende patienter modtog en fast dosis af erlotinib på 150 mg daglig og 4 andre deltager tog en fast dosis på 100 mg daglig mod ikke små cellet lungkræft. Plasmakoncentrationen for erlotinib og dets hoved metabolit, 4-hydroxyerlotinib blev målt og vurderet i blodet ved hjælp af HPLC metode med UV detection.

Resultater

Plasmakoncentrationerne af erlotinib blev beregnet,den ligger i området 0.76-3.63 mg / L hvor den laveste koncentration blev målt hos en patient, som tager den lavere dosis. Ellers afviger plasmakoncentrationerne for de patienter der tager lavere dosis af erlotinib (daglig dosis på 100 mg) ikke fra de andres. Der var kun én ryger i gruppen og hans plasmakoncentration adskiller sig ikke fra de andre patienter i undersøgelsen. De ældre patienter over 70 år viste ikke højere koncentrationer af erlotinib. Den vigtigste metabolit blev dannet i betydelig mængde med steady state situationen varierende fra 0.076 til 0.42. Størstedelen af patienterne var i den lave ende med 3 outliers med et forhold på omkring det dobbelte af andre.

Konklusion

En bioanalytisk metode blev udviklet og valideret til dette cross-sectional undersøgelse om interpatient variabilitet af erlotinibs farmakokinetik. Variationen i dannelsen af den aktive metabolit og steady state koncentration er opnået med en god klinisk effekt. Alle patienter syntes at have en plasmakoncentration over den minimale effektive niveau og alle havde også en betydelig del af 4-hydroxymetabolit dannet i betydelig mængde.

Abbreviations

NSCLC	Non Small Cell Lung Cancer
SCLC	Small Cell Lung Cancer
EGFR	Epidermal Growth Factor Receptor
TKI	Tyrosine Kinase Inhibitor
TNM	Tumor Node Metastases
CT scans	Computed Tomography Scan
PET scan	Positron Emission Tomography Scan
ATP	Adenosine Tri-Phosphate
PK	PharmacoKinetics
ABC transporter	ATP-Binding Cassette transporter
AUC	Area Under the Curve
DMSO	DiMethylSulfOxid
QC	Quality Control
PP	Protein Precipitation
CRF	Case Report Form
E	Erlotinib
M	Metabolite (4-hydroxyerlotinib)
IS	Intern Standard (4- methyl erlotinib hydrochloride)
LLOQ	Lower Limit of Quantification (LLOQ)

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Aim of the study

Erlotinib is a small molecule inhibitor of the epidermal growth factor receptor used for patients with non small cell lung cancer. This study is aimed to find an optimal dose of erlotinib for each individual patient at a dose devoid of toxicity and within the therapeutic range. In order to estimate the person-to-person variability in plasma concentration of fixed dose erlotinib in patients with non small cell lung cancer (NSCLC), erlotinib concentration will be determined in the blood plasma by using a HPLC method and then correlate this to other factors that may influence the drug disposition.

1. Introduction and theoretical background

1.1 Epidemiology

There were approximately 4200 cases of lung cancer annually from 2005 to 2009 in Denmark. The incidence has been increasing rapidly for men until the mid-80s and for women until the mid-90s. This disease is the cause of approx. 24% of all cancer deaths in Denmark. There are almost as many women who get lung cancer as men. There is an increasing incidence with increasing age. 33% of cases are 75 years or older. Tobacco smoking is the main etiological factor for development of lung cancer, but radon and industrial exposure of asbest can influence as well. The relative risk of developing lung cancer from smoking 20 cigarettes a day is 5000% (compared with non-smoking). Cessation of smoking reduces the risk gradually, at least 5 years of cessation is needed to half the relative risk. The cancer type that often occurs among non-smokers is called adenocarcinoma, which is a malignant tumor originating in glandular epithelium [Den lille onkolog].

Only 15% of all lung cancer patients survive 5 years after diagnosis despite new chemotherapeutic agents. Prognosis of this disease is serious. In the last years efforts have led to the emergence of the new group of TKI as erlotinib and gefitinib in advanced NSCLC [Steins et al., 2010].

1.2 Pathology

Because of differences in the biology and therapy strategy the tumors are divided into small cell lung cancer (SCLC) and NSCLC, which consists of the three other main types as squamous cell carcinoma (40%), adenocarcinoma (40%), and large cell carcinomas (5%). Both the squamous cell carcinoma and SCLC are found most commonly centrally near the large airways, whereas adenocarcinoma is often located peripherally. All these types of lung cancer have a great tendency to spread; regionally to the mediastinal lymph nodes or distant to the rest of the lung, liver, adrenal, bone, bone marrow and brain.

In order to target and find out a suitable treatment of the individual patient different markers/ mutations in the tumor is identified. Currently mutation in Epidermal Growth Factor Receptor (EGFR) exon 19-21 has a major impact on the treatment choice for patients with NSCLC treated in palliative purposes. Patients with these mutations are treated with Tyrosine Kinase Inhibitors (TKI) such as gefitinib or erlotinib, which is a standard treatment [Den lille onkolog].

1.3 Lung cancer

There are two main types of lung cancer NSCLC and SCLC, as they behave differently they are treated differently. Lung cancer symptoms vary from person to person, the most common are cough, cough changed habits, bloody mucus, fatigue, loose of appetite and weight, shortness of breath and hoarseness. Other symptoms of the disease can be pneumonias and chest pain as well. Among smokers the symptom shows as a worsening of the irritation of the respiratory tract problems that they already have.

Diagnosis can be made through endoscopic examinations, taking tissue samples (biopsy), lung radiographs and computed tomography (CT Scans). By using a combination of positron emission tomography (PET) and CT scans it is possible to find out the location and the size of the knot; whether it is close to other bodies and how far the disease has spread to other body organs as the liver and adrenal glands. Blood tests are done for evaluating how well the liver and the kidneys are functioning to make sure that the patient can tolerate the treatment.

The best possible treatment can be offered if it is known what stage the disease is in. Stage classification is used to predict the course of the disease. Lung cancer is divided into stages based on how big the knot is and whether the cancer has spread to lymph nodes or to other organs [Lungekræft, cancer.dk brochure].

1.3.1 Staging of NSCLC

Both types of lung cancer (NSCLC and SCLC) are divided into stages. Since the investigational medicine (Tarceva) in this study is used among NSCLC patients the focus is on NSCLC and to omit a description regarding SCLC´s treatment and stage. NSCLC is divided into four stages based on how much the node is grown into other organs or into the chest wall.
The stage classification depends also on whether there is a spreading to the lymph nodes and other locations in the body (metastasis). The larger the tumor is the more it grows into the surroundings and the higher the stage becomes. Beyond that the more lymph node areas are affected the higher the stage will be. The lung cancer reaches its highest stage when it has led to metastases in other organs [Lungekræft, cancer.dk brochure].

There are two ways of staging lung cancer; the number and tumor node metastases (TNM) staging systems. The number system is divided into four main stages; in stage 1 the cancer is limited and just affects the lung; in stages 2 and 3 the cancer has spread into lymph nodes or other organs close to the lung and in stage 4 the cancer has spread to other body organs.

The TNM staging system takes into account the size of the tumor (T), whether cancer cells have spread into the lymph nodes (N) close to the cancer and whether the tumor has spread anywhere else in the body, which is called a secondary cancer or metastases (M). The doctor gives each of these factors a number.

According to table 1 stage I-II is termed as a local disease where the tumor is located in the lung. Stadium IIIA describes the loco-regional or locally advanced disease, here the tumor is located outside the lung; in other words understood as the surrounding structures or mediastinum. Stage IIIB is considered as advanced disease in case of massive nodal involvement or if the extra-spread makes the curative treatment unrealistic. Stage IV is called advanced disease

Table 1: The two staging systems (number- and TNM staging system) tell the doctor the same thing in a slighty different way. The information below shows how the TNM staging information fits into the number staging system. NSCLC consists of four stages from tumor node metastases (TNM) classification. The staging of the disease depends on the size and the location of the tumor. In practice the disease is classified as specified [National cancer institute; Lungekræft, cancer.dk-brochure; cancer research UK].

Number system	**TNM staging system**	**Number system**	**TNM staging system**
(1)IA	T1a, b N0	**(2)IIB**	T2b N1 - T3 N0

(1)IB	T2a N0	**(3)IIIA**	T1a, b, T2a, b N2 - T3 N1, N2 - T4 N0-1
(2)IIA	T2b N0 - T1a,b N1 - T2a N1	**(3)IIIB**	T4 N2 – all T N3
(4)	Stage 4 lung cancer is TNM stage – Any T, any N, with M1A or M1B.		

1.3.2 Erlotinib (Tarceva) and its mechanism of action

Approximately 20 percent with NSCLC are being operated in Denmark. NSCLC patients will undergo surgery, if it is possible, most of them will get chemotherapy afterwards. If it is not possible to operate chemotherapy and/or radiation therapy will be offered [Lungekræft, cancer.dk brochure].

Erlotinib hydrochloride is a protein kinase inhibitor, an antineoplastic agent and the active substance of Tarceva, which is used to treat advanced stage of NSCLC and pancreatic cancer.

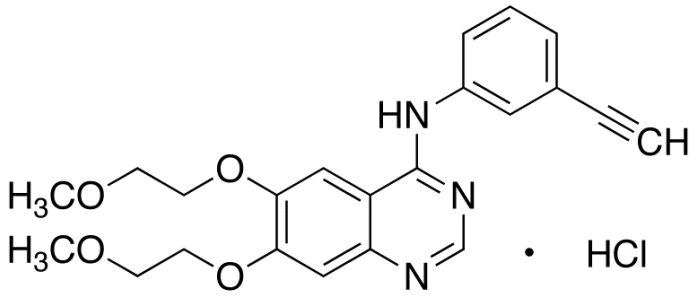

Figure 1: Structure of Tarceva (Erlotinib Hydrochloride)/ OSI-774[HIDALGO AND BLOEDOW; 2003].

This project deals with NSCLC patients only. Erlotinib is a reversible tyrosine kinase inhibitor that specifically targets the epidermal growth factor receptor, which is sometimes highly expressed and occasionally mutated in various forms of cancer. In the treatment of cancer the role of protein kinase inhibitors are to inhibit one or more of the protein kinases that are important for the growth and proliferation of the malignant cells and/ or angiogenesis in the tumor.

Erlotinib binds in a reversible fashion to the adenosine triphosphate (ATP) binding site of the receptor [Raymond et al., 2000]. For the signal to be transmitted, two membrane receptors of the EGFR family need to come together to form a homodimer. These use ATP to autophosphorylate each other, which causes a conformational change in their intracellular structure, exposing a further binding site for proteins that cause a signal cascade to the nucleus. By inhibiting the ATP,

autophosphorylation will not be possible and the signal cascade will be stopped. Erlotinib has been registered for use in NSCLC at a fixed dose of 150 mg/day. This dose was chosen based on phase 1 data, where the dose limiting toxicity was observed at 200 mg/day in a small patient sample [Gordon et al., 2005; Karp et al., 2000]. Fixed dosing may result in suboptimal treatment or excessive toxicity because of high inter-individual variability in the pharmacokinetics (PK) of these therapies.

1.3.3 Erlotinib (Tarceva) pharmacokinetics

Erlotinib is used by patients suffering from NSCLC and in combination with gemcitabine by metastatic pancreatic cancer patients. It is given orally to NSCLC patients; the dosage suggestion is 150 mg erlotinib daily for adult patients according to the product label [medicin.dk]; the drug should be taken at least 1 hour before or 2 hours after food intake.

The drug is metabolized by CYP3A4, CYP1A2 and CYP1A1. Caution must be taken by simultaneous use of potent inhibitors and inducers of these CYP enzymes. Concomitant use of CYP inducers or inhibitors may change the plasma concentration of erlotinib. For example ketoconazole increases the area under the curve (AUC) of erlotinib by 85% whereas tobacco smoking conversely leads to increased elimination rate of erlotinib probably due to induction of CYP1A2 [medicin.dk; Ling et al., 2006].

Since CYP enzyme inhibitors results in the reduction of erlotinib metabolism and thus increases its concentration in plasma, this can lead to decreased effect. While an inducer of the enzyme increases the conversion and metabolism of erlotinib, this can cause toxicity or elevated side effects. Diarrhea and rash are the most common adverse reactions which sometimes require dose reduction.

Erlotinib has a bioavailability of about 60% which is increased by concomitant food intake. After 7-8 days the steady state will be achieved. It has a plasma half-life of approximately 36 hours. It is metabolized in the liver by cytochrome enzymes. The main metabolites of the drug are pharmacologically active and are eliminated via feces [medicin.dk].

Erlotinib is widely distributed throughout the body after oral administration. It is metabolized in the liver by cytochrome P450s, primarily by CYP3A4 and CYP1A2, CYP1A1. CYP3A5 also plays a minor role in erlotinib metabolism (figure 2). In addition to its role as an ATP-binding cassette transporter (ABC transporter) inhibitor, erlotinib has also been implicated as an inhibitor of CYP2C8 and UGT1A1 activity. The UGT1A1 gene belongs to a family of genes that provide

instructions for making enzymes called UDP-glucuronosyltransferases, UGT1A1 gene is located on chromosome 2). Hence erlotinib may inhibit the metabolism of co-administered drugs that are substrates of CYP2C8 and UGT1A1 [Erlotinib pathway pharmacokinetics, 2012; McDonagh 2011].

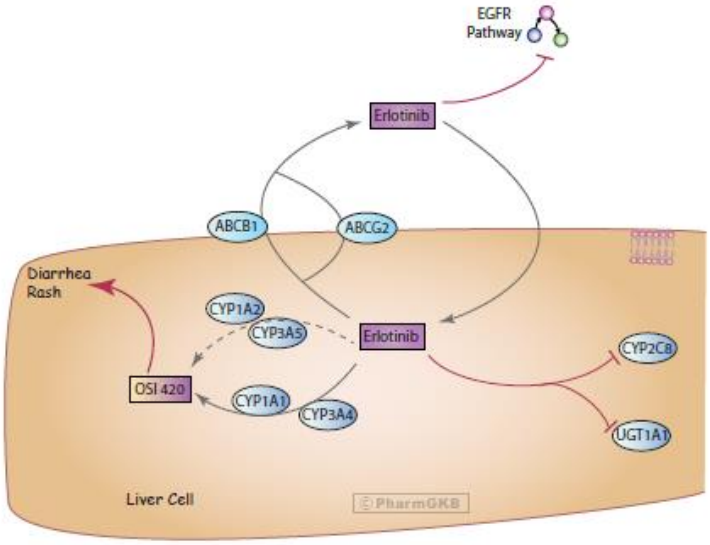

Figure 2: Erlotinib disposition, a human liver cell model; showing genes involved in the transportation and metabolism of erlotinib. Erlotinib is a synthetic anilinoquina-zoline compound that selectively binds to the ATP-binding site of the epidermal growth factor receptor (EGFR)-TK, which inhibits receptor tyrosine kinases (TKs). Erlotinib also inhibits ATP-binding cassette transporter-mediated drug efflux, which in turn strongly increases the intracellular concentrations of co-administrated drug molecules that are transporter substrates [Erlotinib pathway pharmacokinetics, 2012].

Erlotinib will lose pharmacological activity during phase I oxidative or reductive reactions. Phase I products or parent drugs are conjugated through phase II reactions that usually form inactive polar products readily available for renal and biliary elimination [klümpen et al., 2011, Ling et al.,2006].

1.4 Experimental background and purpose

The hypothesis of this project is that erlotinib is given as a fixed dose of 150 mg to all patients, which results in a variable plasma concentration of the drug. This would in turn lead to a variation in anticancer effect and toxicity. The size of person-person variability will be determined in this project. Should a sizeable difference be found, it would indicate the need for further studies of potential differences in anticancer activity and toxicity and a reassessment of drug dosage. The perspective of this study is that it may lead to optimized treatment of NSCLC.

Tyrosine kinase inhibitors such as erlotinib are a class of drugs available for cancer treatment. Fixed dosing is still standard practice, even though inter-patient variations are likely and well known from classical chemotherapy, due to variability in exposure caused by variation in

bioavailability of oral drugs. The reason for this variability is different absorption, distribution, metabolism and excretion (ADME) processes. They have the influence on the kinetics of drug exposure to the tissue. The identified factors that have an influence on drug disposition are genetic polymorphisms, age, gender, diet, smoking, alcohol consumption, renal and liver function, concomitant diseases and co-medication [klümpen et al., 2011].

Erlotinib is used for advanced non-small cell lung cancer in patients who have already been treated with at least one other chemotherapy regimen (called second line), and in first line to patients with a known EGFR mutation. It works by blocking the action of an abnormal protein that signals cancer cells to multiply. Erlotinib helps to slow or stop the spreading of cancer cells by inhibition of a varity of biological processes such as cell growth, differentiation and resulting in cell-death; apoptosis [nih.gov, 2012].

As mentioned previously, erlotinib is a TKI of the human EGFR type 1/EGFR (HER1/EGFR) as it possesses a clinical antitumor effect and prevents the intracellular phosphorylation of tyrosine kinase associated with the EGFR.

Erlotinib, as well as other small-molecule anticancer drugs, is approved at a fixed dose. Association between certain toxicities and treatment efficacy has been demonstrated such as skin rash that might be used as surrogate marker for effect [Drugdex, 2012; Pérez et al., 2004; Reck et al., 2010]. Alternatives to fixed dosing have been explored such as Therapeutic Drug Monitoring, genotype or phenotype adjusted dosing or dose adjustment according to toxicity. In early development of erlotinib the dose limiting toxicities was observed at 200 mg/day and a dose of 150 mg/day was adopted as the maximal tolerated dose used in all subsequent studies.

An important observation in the preclinical studies with erlotinib was the direct relationship between target inhibition and antitumor effects in the animal model, suggesting that sufficient doses to inhibit the target in tumor tissues would need to be administered for this agent to be effective in the clinic [Karb et al., 2000].

In summary, the following points to several reasons for a large inter-individual variation in PK of erlotinib:

- Absorption and bioavailability is increased by food. It is advised that patient should take the drug without food, but variations in stomach emptying will have an influence on residual food and thus on absorption.
- Metabolism by cytochrome P subtypes CYP3A4, CYP1A2 and CYP1A1, the activities of which are determined to various extents by heritage.
- Interaction with other drugs metabolized with the above enzymes.
- Presence of biologically active metabolites which kinetics and potency may vary among patients.
- Long elimination half-life and low clearance which is a particular problem in patients with impaired liver function and high age.
- A large variation in the volume of distribution of erlotinib [medicin.dk].

It is proposed that a fixed dose of erlotinib, due to the ADME factors leads to a variable drug exposure in patients. As binding in the ATP pocket is reversible and happens in competition with ATP this would in turn lead to variations in effect and side effects. It is possible that TKI´s should be dosed on an individual level.

Dosing of cytotoxic drugs with large inter individual variability should preferably be based on population methods including pharmacokinetics and pharmacodynamics. The whole study on erlotinib optimal dosing will consist of three sub studies in long term; only one (the cross sectional study) of them is investigated in this thesis. Briefly the three sub-studies are defined as:

- *Cross sectional:* Here a single blood sample is taken after a minimum of 7 days of erlotinib treatment to determine the person-person variability.
- *Longitudinal:* Several blood samples for each patient is taken a minimum of 4 weeks apart to determine the variations within the individual.
- *Initial:* Several blood samples are taken with short interval at time of start of erlotinib treatment to investigate ADME factors.

The aim of this cross sectional study is to assess the magnitude of the inter-individual plasma concentration of erlotinib as the first step in this process.

2. Experimental

2.1 Equipment

- High performance liquid chromatography (HPLC); Agilent Technologies 1120 Compact LC.
- Water symmetry C18 reversed phase analytical column (150 mm length x 4.6 mm diameter).
- Standard equipment's as spin, weight ,pH meter, magnetic stirrer and vortex mixer were used.

2.2 Materials

Substances used in the laboratory

- The main substance is the drug Tarceva containing the active compound Erlotinib Hydrochloride; it is also indicated with OSI-774.The drug has the molecular formula $C_{22}H_{24}CLN_3O_4$ and a molecular weight 429.90g/mol. It has according to the analytical information's a melting point from 225 to 2270C. The product is in powder form with an appearance of white solid and a purity of 98%. In long term the substance can be stored at -20 0C in the freezer. This product is soluble in dimethyl sulfoxide (DMSO) and methanol. Erlotinib hydrochloride´s structure is shown in figure 1 [Toronto research chemicals, 2012].
- The free base (Desmethyl Erlotinib) also called 4-hydroxyerlotinib is one metabolite of erlotinib that is formed in the greatest extent, also written as OSI-420 (see figure 3). This metabolite has the molecular formula $C_{21}H_{21}N_3O_4$ and the molecular weight 379.41 g/mol. Among its analytical information's the melting point is 167-169 0C, the purity is 98% and the product appearance is a white solid. This is also soluble in both DMSO and methanol as erlotinib. The long termed storage is possible in the freezer at -20 0C [Toronto research chemicals, 2012].

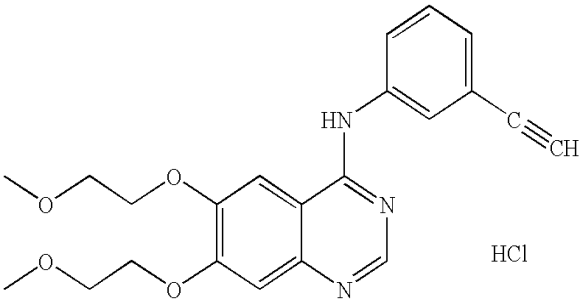

Figure 3: Structural formula of the metabolite; 4-hydroxyerlotinib (OSI-420).

- The internal standard 4-Methyl Erlotinib Hydrochloride (OSI-597) has the structural formula seen on figure 4. It has the molecular structure C23H26CIN3O4 and a molecular weight of 443.93 g/mol. The internal standard is characterized by the following analytical information as a melting point from 227 to 229 0C, a pale pink solid appearance, a purity of 98%, a storage condition at -20 ^{0}C in the freezer and it is soluble in DMSO and methanol [Toronto research chemicals, 2012].

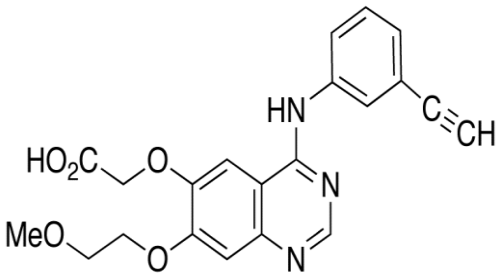

Figure 4: Structural formula of the intern standard; 4-Methyl Erlotinib Hydrochloride (OSI-597).

- In addition to the 3 main substances there were used a number of other substances/chemicals in order to prepare a mobile phase and purify the plasma samples. The solutions and substances are: Pure methanol, pure acetonitrile and blank human plasma; plasma samples containing erlotinib, demineralized water, phosphoric acid (H3PO4) and potassium phosphate (KH_2PO_4) powder with a molecular weight 136.08 g /mol.

Patient population

The study is carried out in patients with non small cell lung cancer (NSCLC), who are treated with erlotinib, regardless of their previous treatment.

The patients have NSCLC and are treated with erlotinib at the Department of oncology; Herlev Hospital. The Patients get both written and oral information (appendix 7) about purpose of the study, method and the advantages and disadvantages of participation.
It is voluntary to participate and the patients can always regret and withdraw the consent without losing their treatment rights. Any patients who give informed consent form (appendix 6) can be enrolled as long as the inclusion and exclusion criteria apply to them. The inclusion criteria apply to patients with a confirmed diagnose of NSCLC; the participants should be 18 years or older who are in or about to start with erlotinib treatment regardless of the disease stage. All patients must receive erlotinib (Tarceva) as a treatment for lung cancer that must be taken at least a week before the sample is taken, so each participating patient must complete a short questionnaire schedule, a case report form (CRF) regarding their other illnesses and medical conditions they might have (appendix 1). The exclusion criteria regard female patients; they should not be pregnant or lactating.

In this cross sectional study blood samples can be drawn at any time after ingestion of erlotinib, but an accurate time of ingestion and sample must be recorded. In the patient´s case record form (CRF) a number of patient characteristics are recorded as height, weight, smoking history, medication and results of standard blood chemistry.

2.3 Methods and study design

Blood sampling

A single blood sample (about 13ml) will be drawn from each of the participating patients. The plasma will be separated and then the samples are stored at -20 ^{0}C for later analysis. The blood samples can be drawn at any time after ingestion of erlotinib, but an accurate time of ingestion and sample will be recorded.

Sample treatment and storage conditions

The samples are centrifuged and labeled, the plasma is separated and stored at -20 ^{0}C until analyzed by HPLC with UV detection [Zhang et al., 2005] .Studies have shown stability for erlotinib and its main metabolite OSI-420 in plasma stored under these conditions in an excess of

1 year [Hamilton et al., 2006]. Naïve plasma from untreated subjects is used for preparation of calibration standards and quality control samples. The quality control samples are used for method validation.

Analysis method for erlotinib

The content of the study entails planning and authority applications of the clinical trial, development and validation of analytical method of erlotinib and its major metabolite based on HPLC-UV, and integration of results to suggest an optimal dosing of erlotinib. Reverse phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection is used to simultaneously determining plasma concentrations of erlotinib (OSI-774) and its major metabolite (OSI-420) [Zhang et al., 2005].

Chromatographic Conditions

The system consists of LC-10ADvp liquid chromatography system equipped with SPD-10 Avp UV detector. The analytes will be separated on water Symmetry C18 analytical column (150 mm x 4.6 mm i.d., 5 µm particles) and a mobile phase consisting of acetonitrile and 0.05 M potassium phosphate buffer in a ratio of 42:48 v/v, respectively. The pH of the mobile phase mixture is adjusted to 4.8 with 85% phosphorous acid (H_3PO_4). A flow rate was set at 1.0 ml/min and the detector was set at a wavelength of 345 nm [Zhang et al., 2005].

2.4 Protocol over the experimental work

Standard and stock solutions

- Stock solutions for erlotinib (OSI-774) and its metabolite (OSI-420) are prepared separately in duplicate by dissolving 2.0 mg of each drug in 10 mL pure methanol. A concentration of 200 µg/ml is obtained, and then stored at -20 ^{0}C. The difference from the mean peak area in each of the duplicate stock solutions should be within 15%.
- Another stock solution (Internal standard) of OSI-597 is prepared by dissolving 2.0 mg drug in 10 ml pure acetonitrile, it should end with a concentration of 200 µg/ml which is stored at -20 ^{0}C. The internal standard is further diluted with acetonitrile to a final concentration of10 µg/ml [Zhang et al., 2005].

- The stock solutions are further diluted each day with blank human plasma in order to prepare calibration standards for erlotinib (OSI-774) and its metabolite 4-hydroxyerlotinib (OSI-420). According to Zhang et al the following concentration ranges as seen in table 2 are suggested to be used:

Table 2: Concentration ranges of Calibration standards (in ng/mL) of erlotinib and its major metabolite, 4-hydroxyerlotinib.

Erlotinib (OSI-774)	12.5	100	500	1000	2000	3000	4000
4-hydroxyerlotinib (OSI-420)	5	25	100	200	300	400	500

A linear regression method/graph is used as calibration curve.

Validation of the HPLC method

The method validation were performed by preparing quality control (QC) samples of erlotinib and 4-hydroxyerlotinib at low, medium and high concentrations, each concentration is repeated and tested on the HPLC six times to get six chromatograms for each substance at each concentration, for this purpose six vials for each concentration are used to check accuracy and repeatability of the equipment. The prepared QC samples are stored at -20 ^{0}C for the duration of a validation procedure [Zhang et al., 2005].

The selected concentration values should be within the calibration standard curve/concentration ranges. The QC samples are prepared by mixing appropriate amounts of the stock solutions with the blank human plasma to obtain the following concentrations from table 3.

Table 3: Quality control samples of erlotinib and 4-hydroxyerlotinib.

Erlotinib (OSI-774)	1000 ng/mL	2500 ng/mL	4000 ng/mL
4-hydroxyerlotinib (OSI-420)	335 ng/mL	417.5 ng/mL	500 ng/mL

Sample preparation

The protein precipitation (PP) method was used a sample pretreatment. Both the aqueous and the plasma containing samples were treated in the same way as follows:

- 250 µL of each sample is mixed with 5 µL, 200 µg/mL internal standard (4-Methyl Erlotinib Hydrochloride (OSI-597)).
- 500 µL of a pure acetonitrile stored at -20 0C is added.
- The sample is mixed for 15 seconds on a vortex mixer, and then centrifuged at a speed of 15000 x g for 15 minutes.
- The supernatant from the plasma samples is separated from the precipitated protein quantitatively. In aqueous samples of the drug and its metabolite of course no supernatant will be formed since they don't contain protein, but they are centrifuged anyway. After centrifugation an amount of the solution will be used for further processing.
- An equal amount of the supernatant (or the centrifuged drug/metabolite solution in the aqueous sample) and the mobile phase will be mixed. For example 100 µL of the sample is mixed with 100 µL of the mobile phase.
- 10 µL of the prepared mixture is injected into the HPLC [Lankheet et al., 2012].

2.5 Statistics

The plasma concentration of erlotinib will be measured by the methods described above. The results will be depicted in graphs. The standard deviation (SD) will be found by using the calculators function 1-var stats.

3. Results

Analysis of erlotinib and its main metabolite

The bioanalysis of erlotinib and its main metabolite 4-hydroxi erlotinib was a modification of a previous method used for pharmacokinetic studies [Zhang et al., 2005]. The analytes and the internal standard 4-methyl-erlotinib-hydrochloride were well separated in the used chromtogarphic system (Figure 5). The standard curve of erlotinib had a coefficient of variation of 0.99 with a small intercept in the range 1 to 4 µg/mL. The coefficient of variation for the metabolite were less R2= 0.93, even though the 3 best points are used (figure 9), the fourth point (400 ng/mL, Area M/IS 0.5212) in appendix 2.1 was far away from the straight line, therefore, it is removed in order to obtain an appropriate standard curve which can be used for the calculation of the metabolite in the patient's blood plasma (appendix 2.6)

Each stock solution as described above was diluted 10 times with water before being injected into the HPLC in order not destroying the column. The stock solutions were injected both separately and as a mixture consisting of 200 µL of each solution, which resulted in a stable baseline and pure chromatogram peaks for 4-hydroxyerlotinib, erlotinib and the internal standard respectively (figure 5).

The single chromatograms were compared to the mixture. It was a help for recognizing which retention time/peak belongs to which substance.

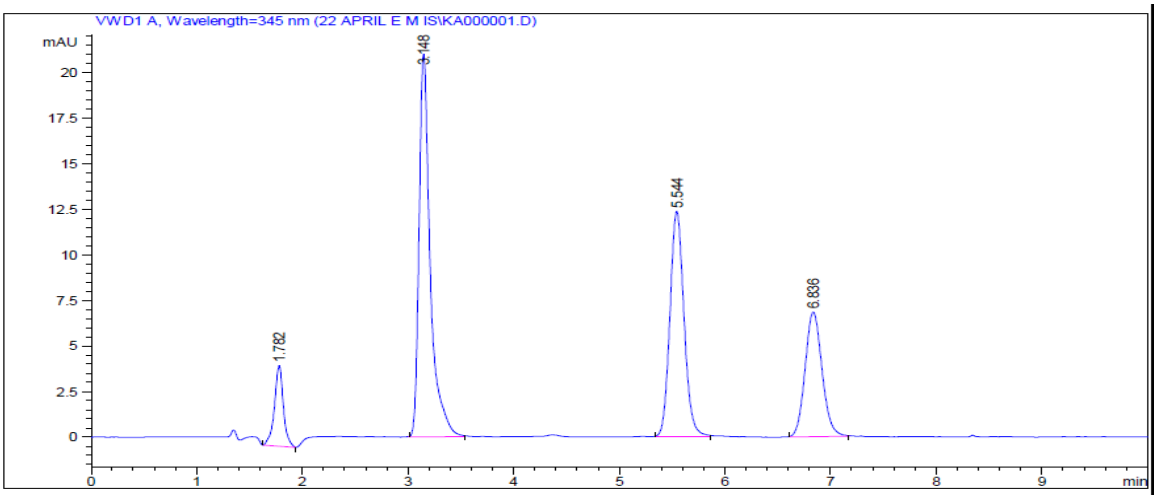

Figure 5: The chromatogram shows three peaks for 3 substances in a solution, which containes 200 µL of each. From the left the three peaks are seen for respectively metabolite (4-hydroxy erlotinib), erlotinib and the internal standard (4-methyl erlotinib).

Next step was making standard curves in water and in plasma. It was desired that the peaks for erlotinib (figure 6) and the metabolite 4-hydroxyerlotinib (figure 7) should not be much lower in plasma compared to water as this is the yield in the validation. The peaks for erlotinib in plasma are similar to that higher than in water in figure 6, whereas the peaks for 4-hydroxyerlotinib is lower in plasma than in water in figure 7. In both figures the points are fairly close to each other even though they are not on a straight line and the correlation coefficient is not that close to 1, especially for 4-hydroxyerlotinib in plasma (figure 7), it can be due to unstable pressure during the measurement or the addition of imprecise volumes of the internal standard to the samples.

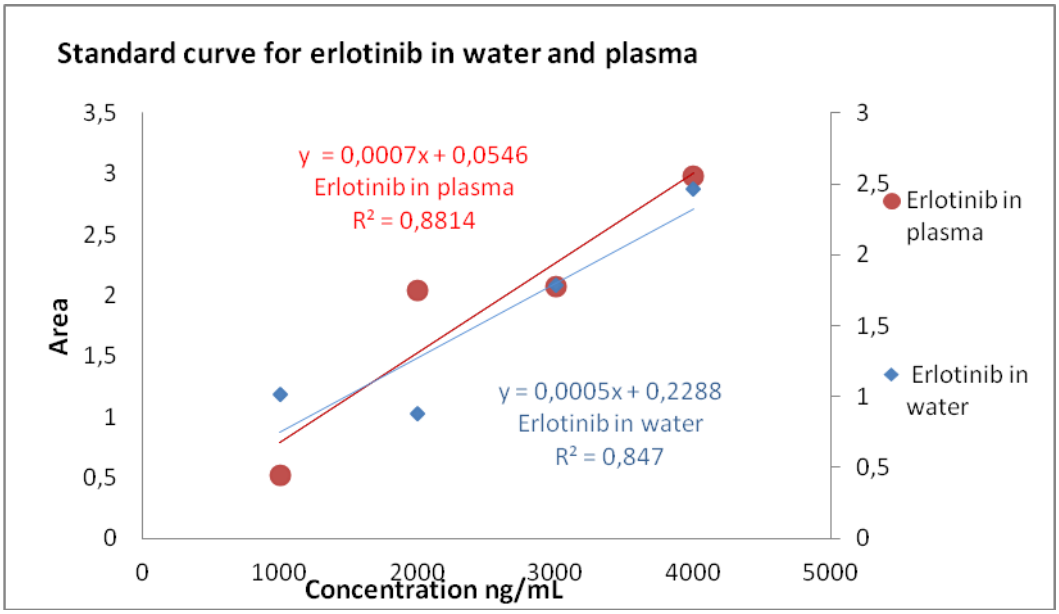

Figure 6: Erlotinib standard curve in both water and plasma (method validation). The area ratio for Erlotinib/IS is plotted as a function of erlotinib concentrations in both plasma and water. In both figures 6 and 7 the values are not far from each other,

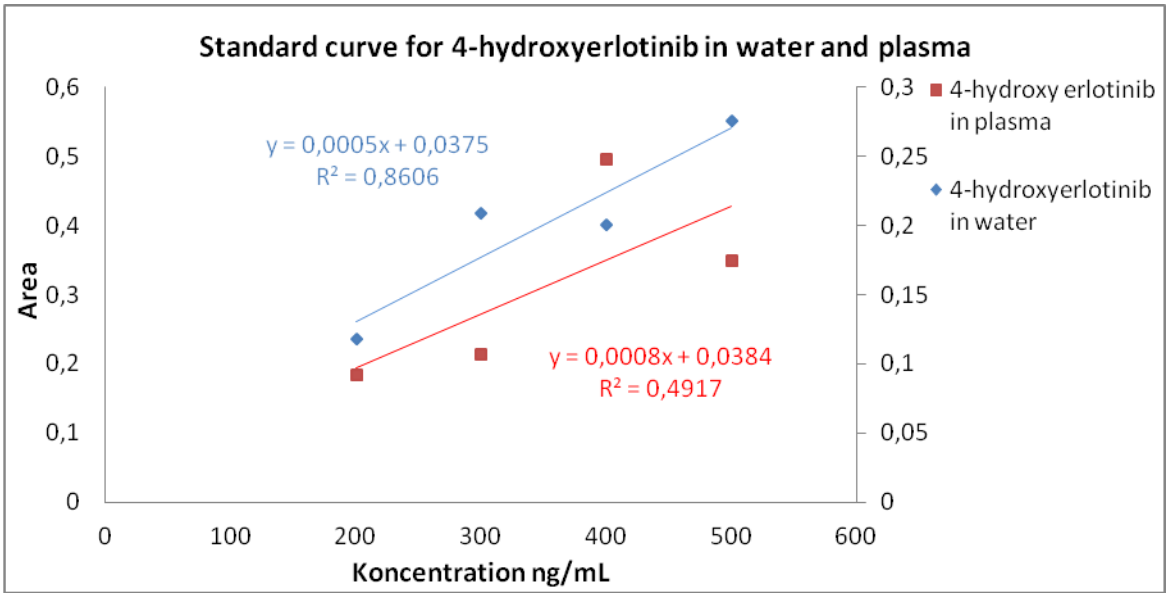

Figure 7: 4-hydroxyerlotinib standard curve in both water and plasma (method validation). The area ratio for 4-hydroxyrlotinib/IS is plotted as a function of 4-hydroxyerlotinib concentrations in both plasma and water.

Precision was tested in several plasma samples with the same added erlotinib and metabolite concentration (Appendix 2.4). It describes the closeness of the individual measurements of an analyte, when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of a biological matrix. Precision can be measured by using a minimum of five determinations per concentration.

At least three concentrations in the expected range are recommended. At each concentration level it should not exceed 15% of the coefficient of variation (CV), but Lower limit of quantification (LLOQ) should not exceed 20% of CV [Bioanalytical method validation]. The precision of the method given as coefficient of variation at three concentration of erlotinib and the main metabolite varied 7.7 to 27% and 9 to 15 %, respectively.

Patient's blood plasma samples were analyzed by the HPLC method. Subsequently, the concentration of both erlotinib and metabolite in the patients plasma were calculated by using a linear regression method/graph calibration curves for both the drug (erlotinib) and its main metabolite (4-hydroxy erlotinib), the graph is consisting of a ratio between the area of erlotinib/IS or 4-hydroxy erlotinib/IS as a function of the concentrations of each substances dilution row (figures 8 and 9).

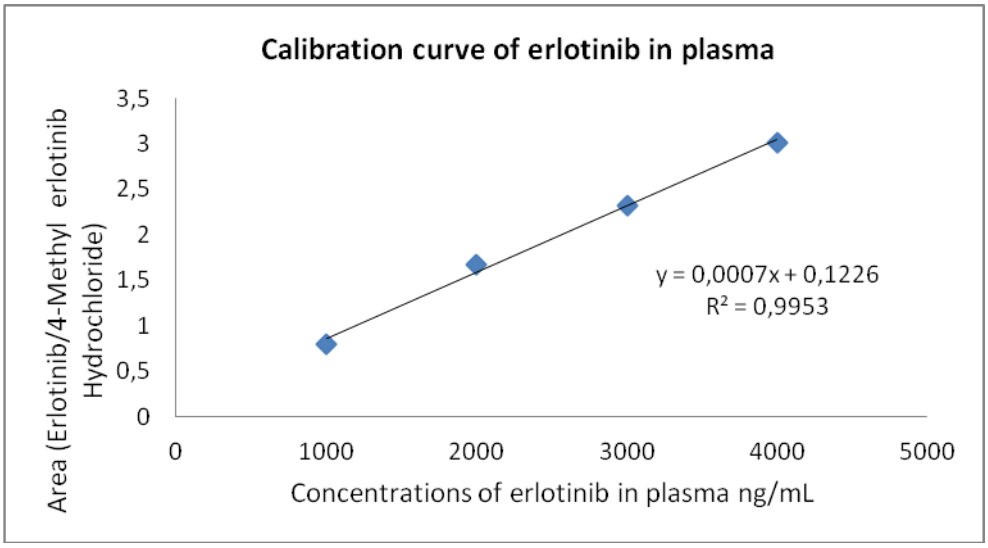

Figure 8: Calibration standard curve of erlotinib. Area(E/IS) is plotted as a function of erlotinib concentration. Is used to calculation of erlotinib concentration among the participating patients.

The equation for the calibration standard curve from figure 8 is used to calculate the drug concentration seen in appendix 2.5. X is the equation of the unknown value, corresponding to the concentrations. Unfortunately, one of the erlotinib plasma concentration and several of the concentrations of the metabolite were below the lowest concentrations in the standard curve.

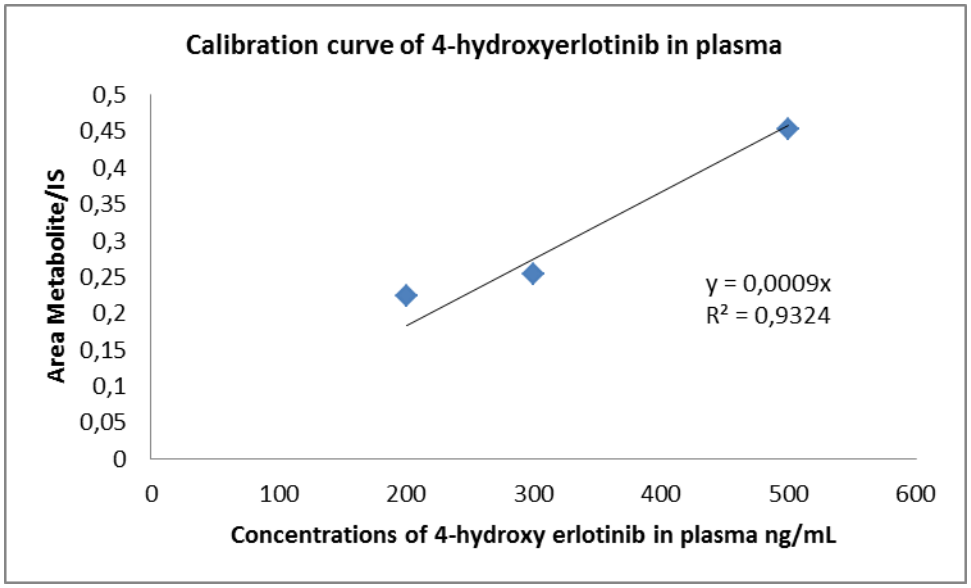

Figure 9: Calibration standard curve of the metabolite 4-hydroxyerlotinib. The area ratio M/IS is plotted as a function of metabolite concentration. Here an outlier/ a point is removed so the curve is based on three most appropriate points to achieve a better curve, which is used for further calculation of metabolite content among the patients.

Plasma concentrations of erlotinib

This cross-sectional study on the interindividual variability of plasma concentrations in steady state of erlotinib and its main active metabolite 4-hydroxyerlotinib has collected 17 subjects. Of these 6 were men and 11 females and the age range was 57-83 year. Their smoking habits as well as co-morbidities and concomitant medication of the patients are seen in appendix 1 and 1.1, where also their ages, and their individual dose of erlotinib can be found. The patient blood sampling was taken at variable times after the administration of Tarceva (erlotinib) after at least one week medication in continuum. The plasma concentrations measured at time after dose is seen in Figure 11. Three patients lacked time of drug administration in the documentation. A tentative time just before blood sampling was used in these cases

The calculated concentrations of erlotinib and 4-hydroxy erlotinib (Appendix 2.5) are plotted as a function of the time the patients took an erlotib tablet to blood sampling; in order to assess how the concentrations are distributed among this patient population.

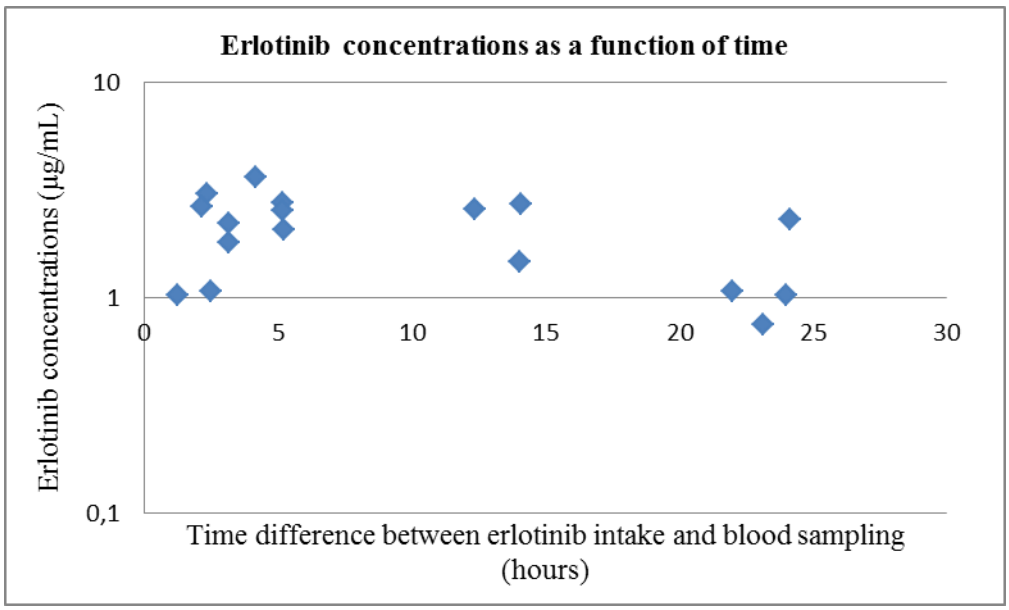

Figure 10: Semi logarithmic curve of 4-hydroxyerlotinib concentrations as a function of the time difference between tablet intake and blood sampling.

The plasma concentrations range of erlotinib were all in the range 0.76 to 3.63 mg/L with the lowest concentration measured in the patient taking the lower dose. Otherwise the patients with the lower erlotinib daily dose of 100mg did not differ from those of the others. There was only

one smoker in the group and his plasma concentration did not differ from other patients in the study. The eldery patients over 70 years of age did not show higher plasma concentrations of erlotinib as compared to the younger. Other differences comparing different patients were looked on for example co-medication and possible drug-drug interactions. Prominent drug-drug interactions was not obvious in any patients in the group except for a tentative pantoprazol inhibition of erlotinib clearance in three patient were two showed higher concentrations as compared to the remaining group (pts 3 and 10)

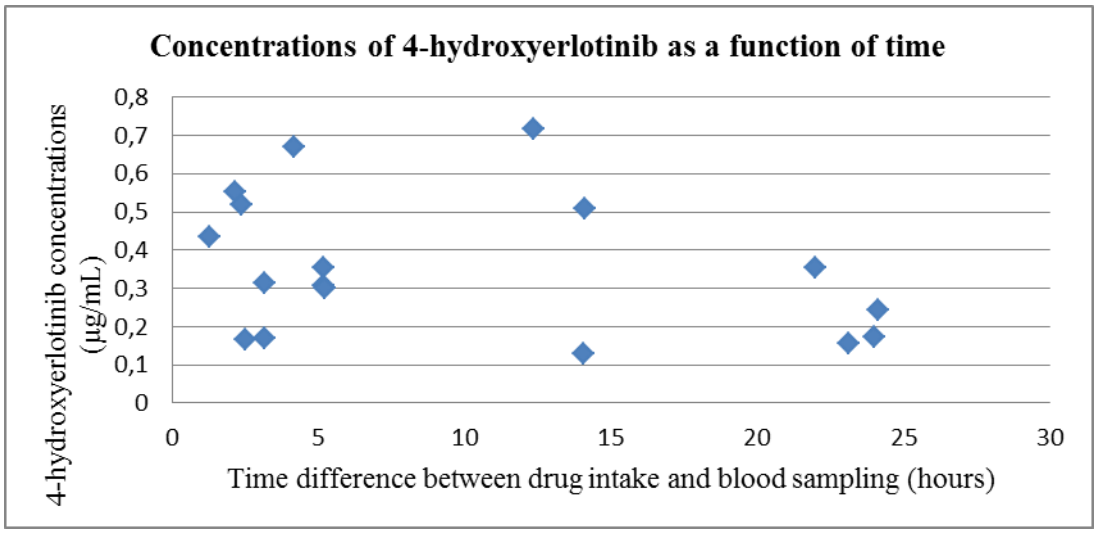

Figure 11: Semi logarithmic curve of 4-hydroxyerlotinib concentrations as a function of the time difference between tablet intake and blood sampling.

The main metabolite was formed in significant amount and the ratio in the steady state situation ranged 0.076 to 0.42. The majority of patients were in the low range with 3 outliers with a ratio of about twice the others (figure 11).

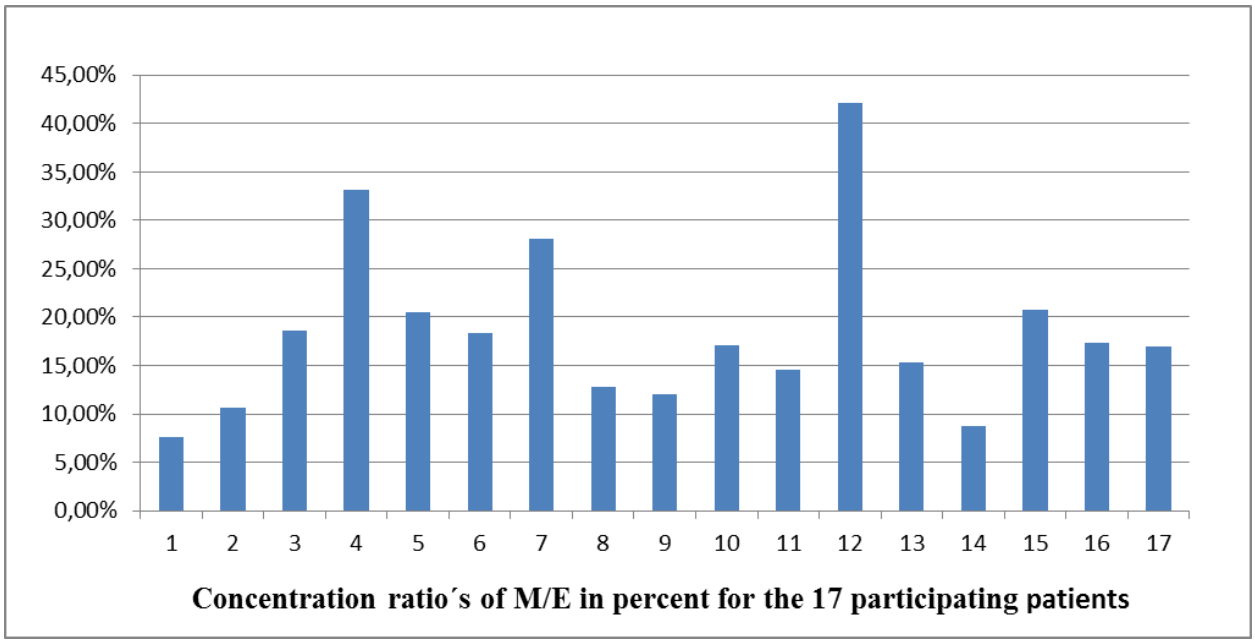

Figure 12: The concentration ratio´s of drug/metabolite are shown in percent for 17 participating patients.

Among the 17 patients number 4, 7 and 12 were more rapid metabolizers of erlotinib since they form the largest amount of metabolite compared to the rest (figure 12).

4. Discussion

The plasma concentrations of erlotinib in the studied patients showed a fairly similar level range and all were above a tentative minimum effective concentration of 0.5 mg/L [Ter Heine et al., 2012]. The plasma sample were not taken at the same time after dose but with low clearance and a resulting long elimination halflife of 36 h [medicin.dk] it must be concluded that all reached a treatment target of erlotinib plasma concentration in the steady state situation. The low range of plasma concentration was a bit surprising regarding the many co-factors determining pharmacokinetics of erlotinib like metabolism of sub-type enzyme enzymes like Cyp 3A4 and Cyp 1A1 of the Cyp P450 system. Cyp 3A4 is generally regarded as monomodal enzyme with no outliers in the ratio of metabolite formed to parent compound. Further variation are caused by influence on bioavailability of food, inducer or inhibitor drugs of the Cyp P450 enzymes given simultaneously and further variation in the volume of distribution. Three patients took other medications along with erlotinib as pantoprazole which is the CYP enzyme inhibitor that may cause a reduced erlotinib metabolism, with increased steady state concentration in two of them [medicin.dk tarceva(erlotinib)], reference Metabolic inhibition is non-selective and might explain the higher plasma concentrations. The difference may tentatively be due on this interaction in patients 3 and 10. Patient number 8 takes Doltard (morphin) and patient number 11 takes cozaar (losartan) which also influence erlotinibs concentration, but with no obvious deviation of plasma concentrations from the group. Even though, almost all the patients metabolizes erlotinib to the main 4-hydroxy-metabolite in significant amounts, except for patient 1 (appendix 2.7).The 4-hydroxyerlotinib metabolite has a claimed clinical effect, but it pharmacokinetics is not known. This may indicate that its concentration may change dramatically even within a dosing interval.

A lot of causes of variability in dose response exists which includes the patient's age, weight, degree of obesity, type and degree of other possible diseases, other drugs concurrently administered (appendix 1) and environmental factors. These factors have influence on the difference in a drugs concentration and metabolism in a population.

The results invites to a population based pharmacokinetic (Pop-PK) study which makes it possible to identify the population patient characteristics that significantly influence the PK parameters, but also take into account pharmacodynamics variance. In general, population based kinetics treats the population rather than the individual patient as the unit of analysis. By doing

so, sparse data from many individuals can be analyzed, and a more representative sample of the target population is obtained.

If we were all alike there would only be one dose strength and regimen of a drug needed for the entire patient population. But we are not alike; we definitely have a great inter-individual variability in response to drugs.

Another important and pervasive cause is genetics, as it is known for many years that only minor differences were observed in the pharmacokinetics and response to drugs between identical twins even when they lived apart and in different social environments, compared with the often experienced wide differences in response within the patient population [Rowland, M., 2011].

The bioanalytical method development was used to establish an assay of erlotinib and its main metabolite and to determine the accuracy, precision, linearity and stability of the analytes in spiked and patient samples. In this study a partial validation is performed due to limited time and limited blanck human plasma. Full validation is important for a new drug entity and erlotinib is already exists in the market, but a partial validation is performed due to modifications of an already validated bioanalytical method [Bioanalytical method validation].

The coefficient of variation (CV=S/mean) is defined as the ratio of the standard deviation to the mean, it shows the extent of variability in relation to mean of the population, it is calculated for each concentrations six area measurement (appendix 2.4) none of them exceed 15% except at the concentration 1000 ng/mL for erlotinib in plasma values, where CV is 27.67%, this lower limit of quantification should not exceed 20%, it can be due to the pressure instability or column might not have been washed enough with 50% acetonitrile solution first and then with mobile phase. Other calculated CV values (in appendix 2.4) are located within the expected range and thus the method is reliable to use.

5. Conclusion

A bioanalytical method was developed and validated for this cross-sectional study on the interpatient variability of erlotinib pharmacokinetics, the variation in formation of a tentative active metabolite and steady state concentration reached for a good clinical effect. All patients seemed to have a plasma concentration above minimum effective level and all also had a significant fraction of 4-hydroxy metabolite formed in significant amount. Factors like age, concomitant drugs intake did not seem to have a major influence on pharmacokinetics. Further

large scale studies on population basis may answer if erlotinib dosing up to 150 mg will give the majority of patient successful treatment of NSCL without harmful effects.

6. Perspectives

Cytotoxic dosing is not straight forward and different bases are used as amount of drug or amount of drug per kg weight of the patient. Others include dosing according to body surface area. Neither of these will give any accurate dose to the tumor since it is forming its own compartment with its own uptake, exposure and pharmacokinetics of the cytotoxic drugs. In best cases it may show some relation to the intensity of side effects. Lately, dosinfg can be based biomarkers from the tumor or based on results from imaging studies. Dosing of cytotoxic drugs with large inter individual variability should preferably be based on population methods including pharmacokinetics and pharmacodynamics. The whole study on erlotinib optimal dosing will consist of three sub studies in long term; only one (the cross sectional study) of them is investigated in this thesis. Briefly the three sub-studies are defined as:

- *Cross sectional:* Here a single blood sample is taken after a minimum of 7 days of erlotinib treatment to determine the person-person variability.
- *Longitudinal:* Several blood samples for each patient is taken a minimum of 4 weeks apart to determine the variations within the individual.
- *Initial:* Several blood samples are taken with short interval at time of start of erlotinib treatment to investigate ADME factors.

The result from the present study invites to a full population based study on erlotinib pharmacokinetics and pharmacodynamics to include the variations that might be implemented in the choice of one dose for all.

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8. Appendixes

Appendix 1: An overview of the Participants CRF

Number? Initials? Sex?	Birth date? Height? Weight?	Do you Work? BMI?	Do you smoke? Do you have other diseases?	What dose erlotinib do you take daily?	Is erlotinib taken at the same time each dag?	Date and time for the last erlotinib intake	Date and time of blood sampling
Patient 1 JEF, male	11.03.47	_____	_____	_____	_____	?	19.11.12 11:15
Patient 2 TFP, male	22.11.30	_____	_____	150 mg	_____	04.12.12 10:15	05.12.12 10:30
Patient 3, ROR, Female	01.04.42, 170 cm, 62 kg	Retired, 21.45	No smoking; Depression	150 mg	Yes	13.12.12 22:00	14.12.12 12:10
Patient 4 JBN, male	19.08.36 80 kg	Retired	No smoking, Hypertension	150 mg	Yes	17.12.12 12:00	18.12.12 10:00
Patient 5 IBL, female	23.02.30 155cm, 80kg	Retired 33.30	No smoking, Osteoporosis Arthritis, Hypertension	150 mg	Yes	17.12.12 12:00	18.12.12 11:15
Patient 6 KJO, female	12.12.52 168 cm, 69 kg	Retired 24.45	No smoking, hypertension	150 mg	yes	18.12.12 8:00	18.12.12 12:15
Patient 7 KHR, female	04.01.50 170 cm, 62.8 kg	Retired 21.73	Don´t smoke, No other diseases	100 mg	Yes	18.12.12 22:00	19.12.12 10:35
Patient 8 VIH, female	05.12.46 159 cm, 52 kg	Retired 20.57	Don´t smoke, no other diseases	150 mg	Yes	27.12.12 6:00	27.12.12 11:17

Patient 9 BIJ, female	12.09.45 164 cm, 70 kg	Retired 26.03	Don´t smoke, Chronic Obstructive Pulmonary Disease (copd)	150 mg	Yes	28.12.12 5:30	28.12.12 10:45
Patient 10 ERK, male	24.05.49 170 cm, 70 kg	Retired 24.22	Don´t smoke,no other diseases	150 mg 10.01.2013	Yes	10.01.13 9:00	10.01.13 11:35
Patient 11 JØS, male	16.06.46 185 cm, 101.5 kg	Retired 29.66	Don´t smoke, no other diseases	100 mg	Yes	07.02.13 6:00	07.02.13 11:20
Patient 12 PEH, male	19.02.56	_____	_____	_____	_____	?	12.02.13 9:25
Patient 13 ALA, female	24.06.50	_____	_____	_____	_____	?	13.02.13 10:50
Patient 14 ALj, female	26.10.46 158 cm, 74 kg	Retired 29.64	Yes 1-2 daily, ulcers and intestinal diverticula	100 mg	No	26.02.13 23:30	27.02.13 13:35
Patient 15 = 8 VIH, female	05.12.46	_____	_____	_____	_____	?	14.03.13 10:15
Patient 16= 7 KHR, female	04.01.50 170cm, 58.6 kg	Retired 20.28	Don´t smoke, no other diseases	100 mg	Yes	18.03.13 7:00	19.03.13 10:15
Patient 17 INM, female	29.08.40 166 cm, 61 kg	Retired 22.14	Don´t smoke, no other diseases	150 mg	Yes	02.04.13 11:10	03.04.13 11:06

Appendix 1.1: Medications in addition of Tarceva (erlotinib)

	Medication	**Dose (mg/ number of times)**
Patient 3, ROR, female Currently taken medications in addition of Tarceva (erlotinib)	Efexor Depot Serequel Prednisolon (induc) Valdoxan Pantoloc Magnesia Symbicort Turbuhaler (PN) Inducer/inhibitor of erlotinib	150 mg x 1 100 mg x 1 50 mg x 1 25 mg x 1 40 mg x 1 500 mg x 2 10 drops, at need Have used the inhibitor pantoprazole (Pantoloc®,Pantoprazol) last month.
Patient 4,JBN, male Currently taken medications in addition of Tarceva (erlotinib)	Selo-Zok Tetracyclin	50 mg x 1
Patient 5, IBL, female Currently taken medications in addition of Tarceva (erlotinib)	Persantin Hjertemagnyl Corodil Hypoloc Simvastatin Petidin (PN)	x 1 75 mg x 1 20 mg x 1 x 1 40 mg x 1 At need
Patient 6, KJO, female Currently taken medications in addition of Tarceva (erlotinib)	Innohep tetracyclin	10000 IE
Patient 7, KHR, female Currently taken medications in addition of Tarceva (erlotinib)	Ibumetin (PN) pantoprazol Gabapentin Doltard Pinex Domperidon Tetracyclin	400 mg at need 40 mg x 1 400 mg x 4 120 + 90 + 120 + 120 mg 14 mg x 4? 10 mg x 3 250 mg x 1
Patient 8, VIH, female Currently taken medications in	Doltard Morphine DAK	3 x 30mg x 2 ½ x 10 mg x 5

addition of Tarceva (erlotinib)		
Patient 10, ERK, male Currently taken medications in addition of Tarceva (erlotinib)	Pantoprazol Amlodipin Kaleorid Diural Apovit	40 mg x 2 5 mg x 1 750 mg x 1 40 mg x 1 1 tablet x 3
Patient 11, JØS, male Currently taken medications in addition of Tarceva (erlotinib)	Cozaar comp Tetracyclin Digoxin	x 2 300 mg x 2 0.25 mg x 1
Patient 14, ALj, female Currently taken medications in addition of Tarceva (erlotinib)	Nitrofurantoin centyl pantoprazole (inhib) symbicort spiriva treo/ panodil	50 mg x 1 x 2 x 1 x 2 x 1 x 1-2
Patient 17, INM, female Currently taken medications in addition of Tarceva (erlotinib)	Pinex Ibuprofen	1 g x 2 200 mg x 2

Appendix 2: Results in table form

Appendix 2.1: Erlotinib and 4-hydroxyerlotinib calibration standards & patient sample results

Erlotinib in plasma				
Concentrations	Mixing ratio	Area ratio (E/IS)	Area (Erlotinib)	Area (4-Methyl Erlotinib HCL)
1000 ng/mL	5 µL/995 µL	0.7913	4.96805	6.26882
2000 ng/mL	10 µL/990 µL	1.6687	11.72007	7.02329
3000 ng/mL	15 µL/ 985 µL	2.3242	15.03114	6.46731
4000 ng/mL	20 µL/ 980 µL	3.0062	20.11305	6.69056

4-hydroxyerlotinib in plasma				
Concentrations	Mixing ratio	Area ratio (M/IS)	Area (4-hydroxyerlotinib)	Area (4-Methyl Erlotinib HCL)
200 ng/mL	5 µL/4995 µL	0.2241	1.56220	6.97155
300 ng/mL	7.5 µL/4992.5 µL	0.2546	1.72453	6.77223
400 ng/mL	10 µL/4990 µL	0.5212	3.53629	6.78464
500 ng/mL	12.5 µL/4987.5 µL	0.4536	3.04951	6.72288

Patient sample experiment data					
Patient nr	Area, E (erlotinib)	Area ,M (4-hydroxyerlotinib)	Area, IS (4-methyl erlotinib HCL)	Area (E/IS)	Area (M/IS)
1	11.52787	1.04183	6.87520	1.6767	0.1515
2	11.36817	1.44309	6.57386	1.7293	0.2195
3	12.85166	2.88134	6.30800	2.0374	0.4568
4	5.99279	2.19175	6.85833	0.8738	0.3196
5	5.04124	1.07697	7.74554	0.6509	0.1390
6	17.13382	3.86421	6.42835	2.6654	0.6011
7	12.48944	4.21280	6.51684	1.9165	0.6464
8	13.89695	2.14873	6.74750	2.0596	0.3184
9	13.28393	1.92284	6.95324	1.9105	0.2765
10	15.69330	3.25445	6.95747	2.2556	0.4678
11	10.19360	1.76365	6.51112	1.5656	0.2709
12	5.74833	2.66221	6.83099	0.8415	0.3897
13	5.53147	0.93528	6.31002	0.8766	0.1482
14	7.95850	0.79905	6.88731	1.1555	0.1160
15	13.00090	3.26202	6.56334	1.9808	0.4970
16	9.10514	1.84899	6.55384	1.3893	0.2821
17	5.08289	0.94498	6.03320	0.8425	0.1566

Appendix 2.2: Standard curve experimental results for erlotinib in water and in plasma, method validation.

Erlotinib in water				Erlotinib in plasma			
Conc.	Area E	Area IS	E/IS	Conc.	Area E	Area IS	E/IS
1000 ng/mL	2.88419	4.82970	1.0193	1000 ng/mL	4.58926	8.75731	0.5240
2000 ng/mL	2.74702	3.12297	0.8796	2000 ng/mL	9.38617	4.60798	2.0369
3000 ng/mL	6.80771	3.80544	1.7889	3000 ng/mL	13.76215	6.63787	2.0733
4000 ng/mL	13.39194	5.44243	2.4607	4000 ng/mL	15.44093	5.18900	2.9757

Appendix 2.3: Standard curve experimental results for 4-hydroxyerlotinib in water and in plasma, method validation.

4-hydroxyerlotinib in water				**4-hydroxyerlotinib in plasma**			
Concentrations	Area E	Area IS	E/IS	Concentrations	Area E	Area IS	E/IS
200 ng/mL	0.85389	7.26495	0.1175	200 ng/mL	1.01800	5.53903	0.1838
300 ng/mL	1.17494	5.61862	0.2091	300 ng/mL	1.46028	6.85407	0.21305
400 ng/mL	1.24230	6.19726	0.2005	400 ng/mL	1.12562	2.26955	0.4960
500 ng/mL	1.93436	7.01249	0.2758	500 ng/mL	2.21658	6.36017	0.3485

Appendix 2.4: QC samples, method validation.

Erlotinib in plasma				**Metabolite in plasma**			
	Area E	Area IS	Area E/IS		Area M	Area IS	Area E/IS
1000 ng/mL	2.74851	5.41639	0.5074	335 ng/mL	1.76335	4.63983	0.3800
	2.39064	2.64074	0.9053		1.07326	3.32426	0.3229
	3.85522	3.65365	1.0552		1.79165	4.83447	0.3706
	1.8887	1.54196	1.2249		0.85672	2.88424	0.2970
	3.37395	2.65853	1.2691		1.84427	5.17285	0.3565
	4.21984	3.68904	1.1439		1.47165	3.19921	0.4600
Mean (E/IS)			1.0176	Mean (E/IS)			0.3645
Standard deviation, Sx (E/IS)			0.2816	Standard deviation, Sx (E/IS)			0.0561
Population standard deviation σx			0.2571	Population standard deviation σx			0.0512
Variation coefficient (CV)= S/mean			27.67%	Variation coefficient (CV)= S/mean			15.39%
2500 ng/mL	5.88648	1.67646	3.5113	417.5 ng/mL	2.18891	5.15590	0.4245
	12.85500	3.92694	3.2735		2.17339	4.57056	0.4755
	9.41800	2.84333	3.3123		0.83526	1.89865	0.4399

	6.54342	2.40425	2.7216		2.31460	5.62344	0.4116
	11.9494	4.26712	2.8003		0.83695	1.75520	0.4768
	10.95482	3.85819	2.8394		2.17931	5.79476	0.3761
Mean (E/IS)			3.0764	Mean (E/IS)			0.4341
Standard deviation, Sx (E/IS)			0.3292	Standard deviation, Sx (E/IS)			0.0388
Population standard deviation σx			0.3005	Population standard deviation σx			0.0354
Variation coefficient (CV)= S/mean			10.70%	Variation coefficient (CV)= S/mean			8.94%
4000 ng/mL	22.50867	4.37580	5.1439	500 ng/mL	3.20369	5.51264	0.5812
	11.88878	2.28253	5.2086		3.40606	4.89002	0.6965
	22.26785	4.63267	4.8067		3.09427	4.39916	0.7034
	13.74388	2.72993	5.0345		3.19943	5.15536	0.6206
	15.52872	3.39268	4.5771		3.26423	5.37925	0.6068
	11.05723	2.56219	4.3155		3.01553	5.24748	0.5747
Mean (E/IS)			4.8477	Mean (E/IS)			0.6305
Standard deviation, Sx (E/IS)			0.3496	Standard deviation, Sx (E/IS)			0.0563
Population standard deviation σx			0.3191	Population standard deviation σx			0.0514
Variation coefficient (CV)= S/mean			7.21%	Variation coefficient (CV)= S/mean			8.92%

Appendix 2.5: Erlotinib concentrations in blood plasma

Erlotinib concentrations for the participating patients calculated by using the equation

$y=0.0007x + 0.1226$ from figure 8.

Patient nr.	Area E/IS	Conc. µg/mL	Patient nr.	Area E/IS	Conc. µg/mL
1	1.6767	2.2201	10	2.2556	3.0471
2	1.7293	2.2953	11	1.5656	2.0614
3	2.0374	2.7354	12	0.8415	1.0270
4	0.8738	1.0731	13	0.8766	1.0771

5	0.6509	0.7547	14	1.1555	1.4756
6	2.6654	3.6326	15	1.9808	2.6546
7	1.9165	2.5627	16	1.3893	1.8096
8	2.0596	2.7671	17	0.8425	1.0284
9	1.9105	2.5541			
			Mean of concentrations for 17 patients		2.0456
			Standard deviation, Sx (E/IS)		0.8482
			Population standard deviation σx		0.8229
			Variation coefficient (CV)= S/mean		41,46%

Appendix 2.6: Metabolite concentrations in blood plasma

The metabolite (4-hydroxyerlotinib) concentrations in the patients blood plasma are found by using the equation (y=0.0009x) for calibration standard curve shown on figure 9.

Patient nr.	Area M/IS	Conc. µg/mL	Patient nr.	Area M/IS	Conc. µg/mL
1	0.1515	0.1683	10	0.4678	0.5198
2	0.2195	0.2439	11	0.2709	0.3010
3	0.4568	0.5076	12	0.3897	0.4330
4	0.3196	0.3551	13	0.1482	0.1647
5	0.1390	0.1544	14	0.1160	0.1289
6	0.6011	0.6679	15	0.4970	0.5522
7	0.6464	0.7182	16	0.2821	0.3134
8	0.3184	0.3538	17	0.1566	0.1740
9	0.2765	0.3072			

Appendix 2.7: Conc. ratio (M/E)

The ratio between the metabolite and erlotinib concentrations shows that some of the patients with high percent values of M/E ratio can metabolize the drug faster due to higher activity in cyp p450 subenzymes. Other values as time of drug intake and concentrations of drug and metabolite is shown on the table as well.

Patient nr.	Erlotinib intake the latest time before blood sampling	Erlotinib Conc. µg/mL	Conc. of 4-hydroxyerlotinib µg/mL	Conc. ratio (M/E)
1	8:00	2.2201	0.1683	7.58%
2	10:15	2.2953	0.2439	10.63%
3	22:00	2.7354	0.5076	18.56%
4	12:00	1.0731	0.3551	33.09%
5	12:00	0.7547	0.1544	20.46%
6	8:00	3.6326	0.6679	18.39%
7	22:00	2.5627	0.7182	28.03%
8	6:00	2.7671	0.3538	12.79%
9	8:00	2.5541	0.3072	12.03%
10	9:00	3.0471	0.5198	17.06%
11	6:00	2.0614	0.3010	14.60%
12	8:00	1.0270	0.4330	42.16%
13	8:00	1.0771	0.1647	15.29%
14	23:30	1.4756	0.1289	8.74%
15	10:15	2.6546	0.5522	20.80%
16	7:00	1.8096	0.3134	17.32%
17	11:10	1.0284	0.1740	16.92%

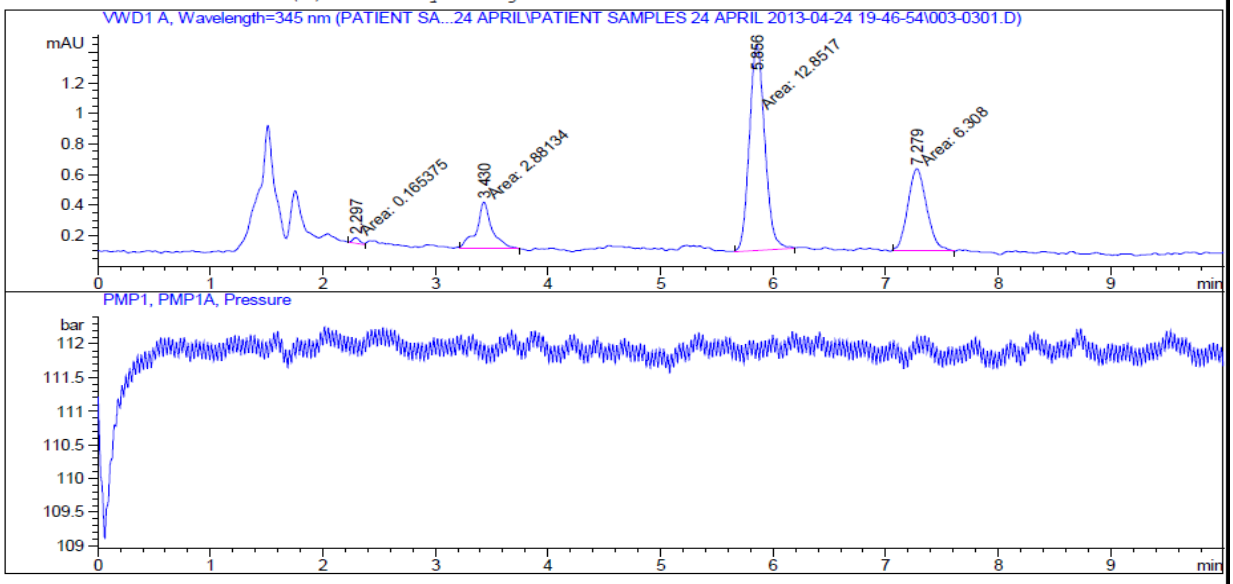
Appendix 3: chromatograms

Chromatogram for patient nr. 3

```
Data File C:\CHEM32\...ES 24 APRIL\PATIENT SAMPLES 24 APRIL 2013-04-24 19-46-54\003-0301.D
Sample Name: Patient 3

======================================================================
Acq. Operator    : ka                              Seq. Line :   3
Acq. Instrument : 1120 Compact LC                   Location : Vial 3
Injection Date  : 4/24/2013 8:35:24 PM                   Inj :   1
                                                  Inj Volume : 10.000 µl
Acq. Method      : C:\CHEM32\2\DATA\PATIENT SAMPLES 24 APRIL\PATIENT SAMPLES 24 APRIL 2013-04-
                   24 19-46-54\ERLOTINIB.M
Last changed     : 4/24/2013 7:43:58 PM by ka
Analysis Method : C:\CHEM32\2\DATA\ERLOTINIB 15 APRIL\ERLOTINIB 15APRIL  2013-04-15 20-29-10\
                   ERLOTINIB.M
Last changed     : 4/22/2013 8:30:13 PM by ka
Method Info      : HPLC with UV detection; Agilent Technologies  1120 Compact LC.
                   Column: C18 waters symmetry, 4.6 mm (d) x 150 mm (L), its particle size is
                   unknown??
                   Flow: 1 mL/min, 10 µL sample is injected into the HPLC, Colonne temp: 30 C
                   Mobile phase A (prepared 04.02.13): 420 mL Acetonitril is mixed with 580 mL
                   ~ 0,05M  KH2PO4.
                   pH is adjusted with PO4- acid to 4,8. The measured pH of the adjusted
                   mixture is 4,57.
                   Mobile Phase B: Nothing/does not exist, since it is isocratic. (MF  A=100%
                   og MF  B= 0%)
                   UV:  345 nm
                   Needle wash: It is performed between each sample by using 50% MeOH (
                   prepared 04.02.13).
                   Tube-, pump- and column wash: 50% acetonitrile mixture is used (prepared
                   19.02.13).


Additional Info : Peak(s) manually integrated
```



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Area Percent Report

==========

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=345 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.297	MM	0.0765	1.65375e-1	3.60456e-2	0.7447
2	3.430	MM	0.1589	2.88134	3.02242e-1	12.9753
3	5.856	MM	0.1592	12.85166	1.34529	57.8737
4	7.279	MM	0.1948	6.30800	5.39741e-1	28.4063
Totals :				22.20637	2.22331	

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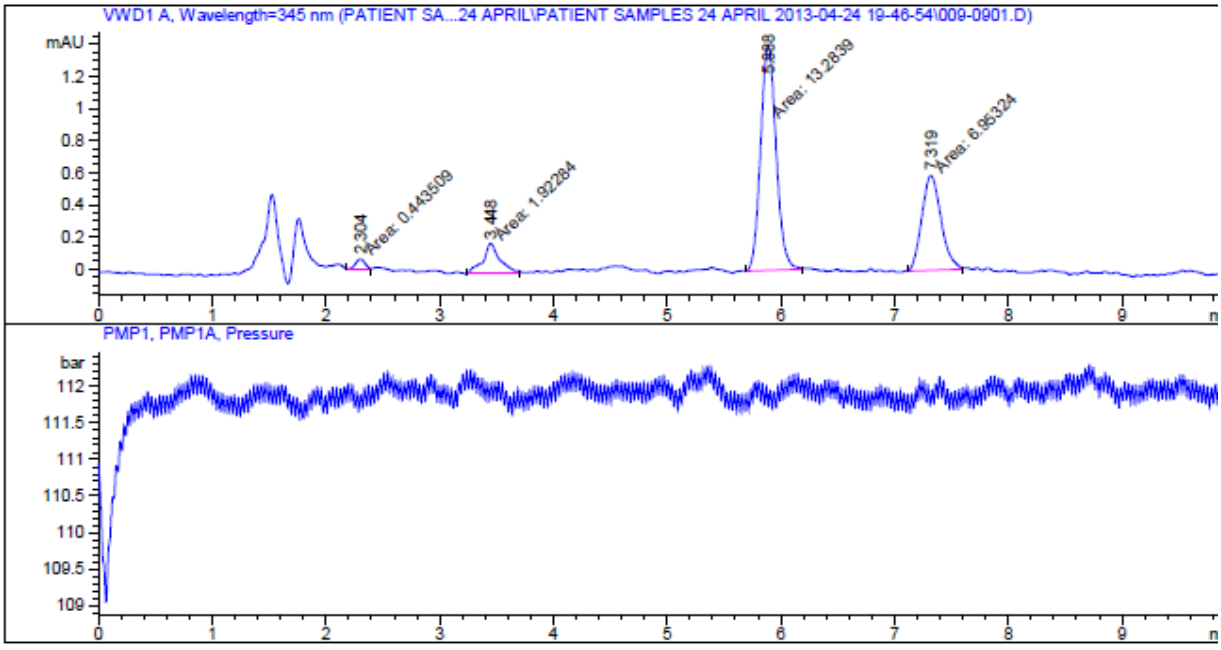
*** End of Report ***

Patient nr. 9 chromatogram

Data File C:\CHEM32\...ES 24 APRIL\PATIENT SAMPLES 24 APRIL 2013-04-24 19-46-54\009-0901.D
Sample Name: Patient 9

```
==================================================================
Acq. Operator   : ka                              Seq. Line :   9
Acq. Instrument : 1120 Compact LC                  Location : Vial 9
Injection Date  : 4/24/2013 10:51:53 PM                 Inj :   1
                                                 Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\2\DATA\PATIENT SAMPLES 24 APRIL\PATIENT SAMPLES 24 APRIL 2013-04-
                  24 19-46-54\ERLOTINIB.M
Last changed    : 4/24/2013 7:43:58 PM by ka
Analysis Method : C:\CHEM32\2\DATA\ERLOTINIB 15 APRIL\ERLOTINIB 15APRIL  2013-04-15 20-29-10\
                  ERLOTINIB.M
Last changed    : 4/22/2013 8:30:13 PM by ka
Method Info     : HPLC with UV detection; Agilent Technologies  1120 Compact LC.
                  Column: C18 waters symmetry, 4.6 mm (d) x 150 mm (L), its particle size is
                  unknown??
                  Flow: 1 mL/min, 10 µL sample is injected into the HPLC, Colonne temp: 30 C
                  Mobile phase A (prepared 04.02.13): 420 mL Acetonitril is mixed with 580 mL
                  ~ 0,05M  KH2PO4.
                  pH is adjusted with PO4- acid to 4,8. The measured pH of the adjusted
                  mixture is 4,57.
                  Mobile Phase B: Nothing/does not exist, since it is isocratic. (MF  A=100%
                  og MF  B= 0%)
                  UV:  345 nm
                  Needle wash: It is performed between each sample by using 50% MeOH (
                  prepared 04.02.13).
                  Tube-, pump- and column wash: 50% acetonitrile mixture is used (prepared
                  19.02.13).

Additional Info : Peak(s) manually integrated
```



Data File C:\CHEM32\...ES 24 APRIL\PATIENT SAMPLES 24 APRIL 2013-04-24 19-4
Sample Name: Patient 9

==========

Area Percent Report

==========

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=345 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.304	MM	0.1049	4.43509e-1	7.04573e-2	1.9621
2	3.448	MM	0.1737	1.92284	1.84537e-1	8.5068
3	5.888	MM	0.1578	13.28393	1.40325	58.7693
4	7.319	MM	0.1960	6.95324	5.91349e-1	30.7618
Totals :				22.60352	2.24959	

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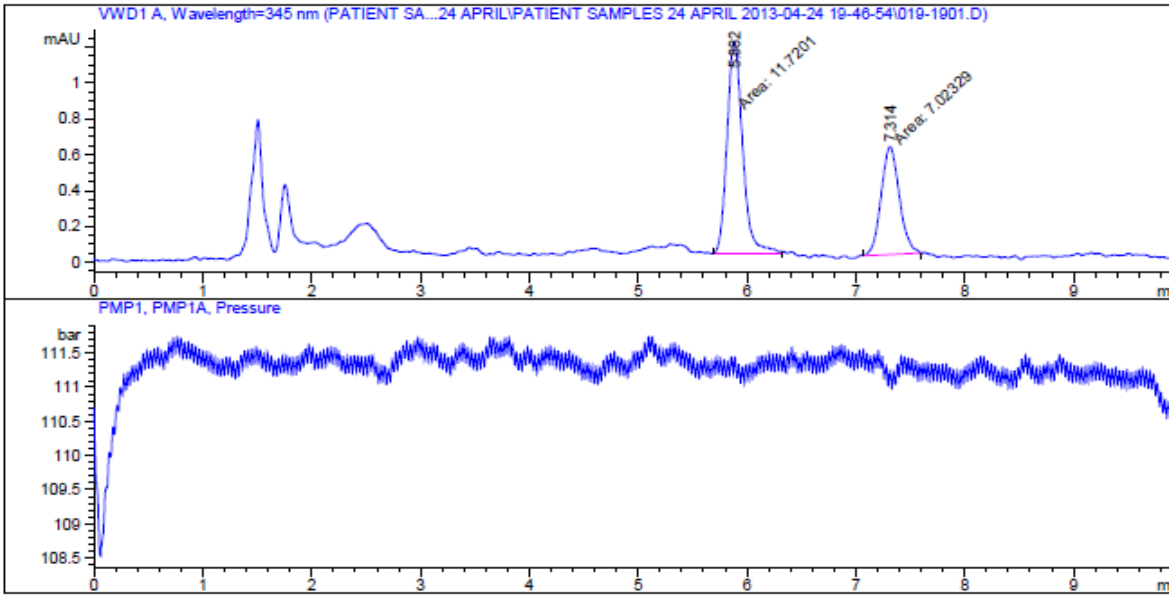
*** End of Report ***

A chromatogram example for erlotinib in plasma, standard curve

Data File C:\CHEM32\...ES 24 APRIL\PATIENT SAMPLES 24 APRIL 2013-04-24 19-46-54\019-1901.D
Sample Name: Erlotinib in plasma, 2000 ng/mL

Acq. Operator	: ka	Seq. Line :	19
Acq. Instrument	: 1120 Compact LC	Location :	Vial 19
Injection Date	: 4/25/2013 2:39:30 AM	Inj :	1
		Inj Volume :	10.000 µl
Acq. Method	: C:\CHEM32\2\DATA\PATIENT SAMPLES 24 APRIL\PATIENT SAMPLES 24 APRIL 2013-04-24 19-46-54\ERLOTINIB.M		
Last changed	: 4/24/2013 7:43:58 PM by ka		
Analysis Method	: C:\CHEM32\2\DATA\ERLOTINIB 15 APRIL\ERLOTINIB 15APRIL 2013-04-15 20-29-10\ERLOTINIB.M		
Last changed	: 4/22/2013 8:30:13 PM by ka		
Method Info	: HPLC with UV detection; Agilent Technologies 1120 Compact LC. Column: C18 waters symmetry, 4.6 mm (d) x 150 mm (L), its particle size is unknown?? Flow: 1 mL/min, 10 µL sample is injected into the HPLC, Colonne temp: 30 C Mobile phase A (prepared 04.02.13): 420 mL Acetonitril is mixed with 580 mL ~ 0,05M KH2PO4. pH is adjusted with PO4- acid to 4,8. The measured pH of the adjusted mixture is 4,57. Mobile Phase B: Nothing/does not exist, since it is isocratic. (MF A=100% og MF B= 0%) UV: 345 nm Needle wash: It is performed between each sample by using 50% MeOH (prepared 04.02.13). Tube-, pump- and column wash: 50% acetonitrile mixture is used (prepared 19.02.13).		

Additional Info : Peak(s) manually integrated



Data File C:\CHEM32\...ES 24 APRIL\PATIENT SAMPLES 24 APRIL 2013-04-24 19-46-54\019-1901.D
Sample Name: Erlotinib in plasma, 2000 ng/mL

Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=345 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.882	MM	0.1653	11.72007	1.18192	62.5292
2	7.314	MM	0.1941	7.02329	6.03044e-1	37.4708

Totals : 18.74337 1.78496

*** End of Report ***

Appendix 4: Letter to Danish Health and Medicine's Authority

Sundhedsstyrelsen

Axel Heides gade 1

2300 S

Ref nr: 2012100805

Variations in plasma concentration in patients with non-small cell lung cancer on fixed dose erlotinib

EUDRACT nr 2012-004350-29

Tak for brev om indsigelse.

Jeg har:

1) Tilføjet styrke på erlotinib idet alle patienter får standard behandling. Forsøget består udelukkende i at der tages en blodprøve til bestemmelse af erlotinib
2) Type of IMP er anført (kemisk)
3) Behandlingen på virkes ikke af forsøget og patienter får den almindelige standard medicin så der anvendes ikke særlig emballage.
4) MedDra er tilføjet
5) Protokollen har fået tilført en sætning i in/ex kriterier mht at udelukke gravide/ammende/fertile kvinder

6) Data på koordinerende investigator er tilføjet
7) Dato på monitor er tilføjet
8) Det er taget til efterretning at SUSARS anmeldes indenfor 7 dage hvis dødelige eller livstruende. I andre tilfælde indenfor 14 dage
9) Det forventes at sidste patient indgår 1 august 2014

Jeg vedlægger: Ny version af protokol og Anmeldesskema

Såfremt styrelsen ønsker hardcopy tilsendt vil jeg gerne have besked.

Vh/ Anders Mellemgaard, Ovl PhD

Appendix 5: Letter to the research ethics committee

Videnskabs etisk kommite

Kongens vænge 2

3400 Hillerød

23-10-12

Ref # h-2-2012-124

Variations in plasma concentration in patients with non-small cell lung cancer on fixed dose erlotinib

Tak for brev af 11/10 2012.

Jeg har foretaget flg rettelser:

1-11: Dokumentation for id, uddannelse er vedlagt, idet jeg kan tilføje at jeg er overlæge ved onkologisk afdeling, ansvarlig for behandling af lungecancer patienter.

Forsøget er anmeldt til Sundhedsstyrelsen Journal no: 2012100805

14: Protokol resume er tilrettet.

17: Jeg har fjernet afsnit om biobank idet dette kan virke meningsforstyrrende. Der vil ikke blive oprettet en biobank men blodprøver vil blive gemt i op til 2 år for at blive analyseret samlet.

18: Man kan ikke lave formel statistik beregning da der er tale om et observations studie med en ukendt fordeling. Der foreligger ikke data fra andre kilder som kan angive forventet standard deviation.

21: På samtykke ark er nu tilføjet særing om journal opl.

24: Præcisering af offentliggørelse i protokol

25: Den etiske vurdering er udbygget

26: Formulering om samtale er udbyget

31-43: Tekstmæssige ændringer og "før du beslutter dig" er vedlagt

Jeg vedlægger version 1.1 af protokol, deltagerinformationer, samtykke ark, protokolresume. Og håber at alt nu er klar til behandling.

Mvh/ Anders Mellemgaard

Appendix 6: Patient Consent Form

Samtykkeerklæring

Jeg giver hermed mit informerede samtykke til deltagelse i undersøgelsen:

Er der forskel fra person til person i koncentrationen af erlotinib i blodet?

Et prospektivt observationsstudie

Navn: _____

CPR: _____

Jeg har fået skriftlig og mundtlig information om undersøgelsens formål og metode, samt fordele og ulemper ved at sige ja til at deltage.

Jeg ved at det er **frivilligt** at deltage, og at jeg altid kan trække samtykke tilbage uden at miste mine nuværende eller fremtidige rettigheder til behandling.

Jeg giver samtykke til at deltage i undersøgelsen og til, at min blodprøve tages og gemmes i op til 2 år. Jeg giver samtykke til at oplysninger om min sygdom og behandling indsamles på baggrund af min journal.

Jeg har fået en kopi af denne samtykkeerklæring samt en kopi af den skriftlige patient information om projektet til eget brug.

Underskrift _____ Dato _____

Erklæring fra den læge, der informerer om forskningsprojektet:

Jeg bekræfter at have informeret mundtligt og skriftligt om undersøgelsen og at der har været mulighed for at stille spørgsmål. Efter min overbevisning er der givet tilstrækkelig og fyldestgørende information om projektet, således at der kan træffes beslutning om deltagelse i forsøget på et kvalificeret grundlag.

Informerende læges navn: _____

Underskrift: _____ Dato: _____

Appendix 7: Participant information

Annex 12.2 Deltager information

Er der forskel fra person til person i koncentrationen af erlotinip i blodet?
Et prospektivt observationsstudie

For personer som skal til at begynde behandling med erlotinib

INDLEDNING

Vi vil med denne informationsskrivelse gerne fortælle om undersøgelsen: *Er der forskel fra person til person i koncentrationen af erlotinib i blodet?* og spørge, om du ønsker at deltage i undersøgelsen.

Denne patient information er beregnet til at give dig oplysninger angående undersøgelsen (som består i at få taget ekstra blodprøver); dine rettigheder i forbindelse med forsøget samt fordele og risici forbundet med forsøget.

Der henvises i øvrigt til det vedlagte bilag "Forsøgspersonens rettigheder i et biomedicinsk forskningsprojekt".
Inden du beslutter, om du vil deltage i dette forskningsforsøg, bør du kende nok til undersøgelsens fordele og risici, så du kan træffe din beslutning på informeret grundlag (Informeret Samtykkeerklæring).
Du er meget velkommen til at stille de spørgsmål, du måtte have til undersøgelsen, og du er velkommen til at tage et familiemedlem, en ven eller en bekendt med på råd inden du beslutter dig.
Når du ved, hvad undersøgelsen handler om og indebærer for dig, vil du, hvis du vælger at deltage i forsøget, blive bedt om at underskrive formularen til Informeret Samtykke. Du har ret til en kopi af dette dokument, som du kan beholde til eventuel senere brug. Endvidere beder vi om tilladelse af Sundhedsstyrelsen kan se i din journal hvis de ønsker at foretage et kontrol besøg.

BAGGRUNDSOPLYSNINGER OG FORMÅL
Grunden til at vi kontakter dig er at du skal til at begynde med behandling med erlotinib (Tarceva).

Stort set alle patienter får samme dosis erlotinib (Tarceva) på trods af at der kan være store forskelle i højde, vægt, lever og nyrefunktion og andre forhold som kan have indflydelse på erlotinib koncentrationen i blodet.

Man ved at virkningen af erlotinib varierer fra person til person, og med denne undersøgelse ønsker vi at finde ud af hvor meget variation der er i den mængde af erlotinib man kan måle i blodet (koncentrationen).

Da vi ikke i dag ved om der er en sammenhæng mellem koncentration og virkning vil den blodprøve vi tager på dig ikke blive brugt i forbindelse med din behandling. Formålet med forsøget er derfor at få mere viden om omsætning af erlotinib i kroppen og af den vej måske blive bedre til at bruge medicinen bedst muligt.

FORSØGSPROCEDURER
Hvis du beslutter dig for at deltage i dette forsøg skal der tages i alt 6 blodprøver med følgende mellemrum efter den første erlotinib tablet:

Samtidigt med første tablet	1 time senere	2 timer senere	24 timer senere	48 timer senere	120 timer senere
Blodprøve 1	Blodprøve 2	Blodprøve 3	Blodprøve 4	Blodprøve 5	Blodprøve 6

Ved hver blodprøve vil du få taget 1 glas af 7-8ml blod. I alt tager vi alt omkring 40 ml blod. Blodprøverne vil blive taget i Onkologisk ambulatorium. Herudover vil vi registrere nogle oplysninger om dig (vægt, højde, medicin, rygevaner)..

Blodprøverne vil blive opbevaret i Klinisk Biokemisk Afdeling, Herlev Hospital, indtil de vil blive analyseret på Analytisk Kemi Institut A på Det Farmaceutisk Fakultet, Københavns Universitet.

Vi forventer, at prøverne vil blive opbevaret i maksimalt tre år. Det biologiske materiale vil ikke blive benyttet til andre projekter eller forsøg. Materiale, der er til overs, vil blive destrueret, når analyseperioden er overstået.

De oplysninger vi indsamler som led i undersøgelsen vil være omfattet af Datatilsynets regler og opbevaret på en sikker måde.

Vi regner med at 5 personer skal deltage i undersøgelsen.

BIVIRKNINGER, RISICI, KOMPLIKATIONER OG ULEMPER
Som tidligere nævnt består undersøgelsen i en ekstra blodprøve. Der udtages ca 40 ml blod i alt, og dette vil ikke komme til at påvirke din blodprocent, eller dit helbred i øvrigt. I forbindelse med blodprøvetagningen kan man mærke et stik, en kortvarig smerte, og nogle kan efterfølgende få et blåt mærke.
Forsøget handler udelukkende om at tage den ovennævnte blodprøve. Alle andre forhold omkring din behandling og kontrol vil foregå fuldstændigt som vanligt.

UDELUKKELSE FRA OG AFBRYDELSE AF FORSØG
Det er vigtigt, at du husker, at det er **frivilligt** at deltage i undersøgelsen og at du kan fortryde dit tilsagn når som helst. Din deltagelse i undersøgelsen vil ikke influere på dit forhold til Onkologisk Afdeling eller din behandling.

OPLYSNINGER OM ØKONOMISKE FORHOLD
Projektet er opstartet af forsøgsansvarlig overlæge Anders Mellemgaard. Der er ingen i projektgruppen, der modtager økonomisk støtte af nogen slags i forbindelse med forsøget. Derfor vil ingen have indflydelse på udførelsen af studiet eller adgang til resultaterne. Ingen af forskerne har økonomiske interesser i undersøgelsen, og der er ingen økonomisk gevinst for afdelingen eller dets personale.

Der udbetales intet vederlag for deltagelse i undersøgelsen.
Du vil være dækket af hospitalets forsikring, hvis der mod forventning skulle opstå skade forårsaget af forsøget.

ADGANG TIL FORSØGSRESULTATER

Forsøget anses som afsluttet efter den sidste forsøgsperson har fået taget den sidste blod og alle analyser er gennemført. Dette forventes at være i løbet af 2015.

Herefter vil forsøgsresultaterne blive analyseret, og positive som negative resultater vil blive offentliggjort.

Vi håber, at du med denne information har fået tilstrækkeligt indblik i, hvad det vil sige at deltage i undersøgelsen, og at du føler dig rustet til at tage beslutningen om din eventuelle deltagelse. Hvis du vil vide mere om forsøget, er du meget velkommen til at kontakte nedenstående kontaktpersoner.

Med venlig hilsen

Overlæge Anders Mellemgaard**,**

Onkologisk afd. R, Herlev Hospital

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Professor Per Hartvig Honoré, Københavns Universitet (Tlf. 3533 6575)

Appendix 8: Application to research ethics committee and Danish Health and Medicine's Authority

Variations in plasma concentration in patients with non-small cell lung cancer on fixed-dose erlotinib

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Other participants in the project

Professor Per Hartvig Honoré, School of Pharmaceutical Sciences, University of Copenhagen.

Stud. Pharm., Khanda Amin, School of Pharmaceutical Sciences, University of Copenhagen.

Variations in plasma concentration in patients with non-small cell lung cancer on fixed-dose erlotinib

Protokol Version 1,1 Oktober 2012

Table of contents

1. Summary

Aim of the study:

In order to estimate the person-to-person variability in plasma concentration of erlotinib in patients with non-small cell lung cancer on fixed dose erlotinib, we plan to determine erlotinib concentration in the blood by using HPLC methods and to correlate this to other factors that may influence drug disposition.

Background and Purpose:

Erlotinib hydrochloride is the active substance of Tarceva, which is used to treat advanced stage non small cell lung cancer and pancreatic cancer. This project deals with non small cell lung cancer (NSCLC) patients only. Erlotinib is a reversible tyrosine kinase inhibitor (TKI), that specifically targets the epidermal growth factor receptor (EGFR), which is sometimes highly expressed and occasionally mutated in various forms of cancer. Erlotinib binds in a reversible fashion to the adenosine triphosphate (ATP) binding site of the receptor [Raymond 2000]. For the signal to be transmitted, two membrane receptors of the EGFR family need to come together to form a homodimer. These then use ATP to autophosphorylate each other, which causes a conformational change in their intracellular structure, exposing a further binding site for proteins that cause a signal cascade to the nucleus. By inhibiting the ATP, autophosphorylation is not possible and the signal is stopped. Erlotinib has been registered for use in non small cell lung cancer (NSCLC) at a fixed dose of 150mg/day. This dose was chosen based on phase 1 data, where the dose-limiting toxicity was observed at 200mg/day in a small patient sample. Fixed dosing may result in suboptimal treatment or excessive toxicity because of a high inter-individual variability in the pharmacokinetics (PK) of these therapies.

Patient population:

Patients with NSCLC treated with erlotinib at the Department of Oncology, Herlev Hospital. Any patient who gives informed consent can be enrolled as there are no exclusion criteria.

Three sub-studies are defined as: Cross sectional: a single sample taken after a minimum of 7 days of erlotinib treatment. Longitudinal: several samples for each patient at least 4 weeks apart. Initial: Several samples taken with short interval at time of start of erlotinib treatment. The same individuals can be part of all three substudies. The plasma concentration of erlotinib and known metabolites as well as activity of relevant CYP enzymes will be measured.

Number of patients and statistical analysis:

We plan to include 100 patients in the cross-sectional study, 20 patients in the longitudinal study with a minimum of 2 samples, and 5 patients in the initial therapy study with 6 samples each. The data will be analyzed by population based pharmacokinetic methodology.

Descriptive statistics as standard deviation (SD) method will be used to assess the variation in plasma concentration of erlotinib.

With a population based pharmacokinetic (Pop-PK) study, it is possible to identify the population patient characteristics that significantly influence the PK parameters. In general, population based kinetics treats the population, rather than the individual patient as the unit of analysis. By doing so, sparse data from many

individuals can be analyzed, and a more representative sample of the target population is obtained. It is possible not only to describe the mean tendencies in the population (i.e. the typical values), but also to describe the random effects including variability between subjects, between occasions and within a subject (residual variability). In this way it is possible to calculate models, in which the correlations between relevant population patient characteristics and the PK parameters are described for an entire group of patients. Such population models can then be used to predict the optimal dosage for future individual patients based on the values of the given characteristics for the individual patient. In this case there are presumably other variables than influences the concentration (sex, age, weight, CYP activity etc).

Number of centers: One: Department of Oncology, Herlev hospital.

Inclusion period: 01. October.2012- 01 October 2014

Study duration: 3 years

2. Experiment background and purpose

The hypothesis of the project is that erlotinib given as at a fixed dose of 150mg to all patients' results in a variable plasma concentration of the drug. This would in turn lead to a variation in anticancer effect and toxicity. The project will determine the size of this person-person variability. Should a sizeable difference be found, it would indicate the need for further studies of potential differences in anticancer activity and toxicity and a re-assessment of drug dosage.
The perspectives of the study is that it may lead to optimized treatment of NSCLC.

Tyrosine kinase inhibitors such as erlotinib are a class of drugs available for cancer treatment. Fixed dosing is still standard practice, even though inter-patient variations are likely and well known from classical chemotherapy, due to variability in exposure caused by variation in bioavailability of oral drugs. The reason for this variability is different ADME processes. This abbreviation stands for absorption, distribution, metabolism and excretion. They have an influence on the extent and kinetics of drug exposure to the tissue. The identified factors that have an influence on drug disposition are genetic polymorphisms, age, gender, diet, smoking, alcohol consumption, renal and liver function, concomitant diseases and co-medication.

Erlotinib is used for advanced non small cell lung cancer in patients who have already been treated with at least one other chemotherapy regimen (called second line), and in first line to patients with a known EGFR mutation. This drug belongs to a class of drugs called kinase inhibitors. It works by blocking the action of an abnormal protein that signals cancer cells to multiply. Erlotinib helps to slow or stop the spreading of cancer cells by inhibition of a variety of biological processes such as cell growth, differentiation and death (apoptosis) [nih.gov 2012]. As mentioned previously, erlotinib is a tyrosine kinase inhibitor of the human Epidermal Growth Factor Receptor Type 1/Epidermal Growth Factor Receptor (HER1/EGFR), as it possesses a clinical antitumor effect and prevents the intracellular phosphorylation of tyrosine kinase

associated with the EGFR [DRUGDEX 2012]. Erlotinib selectively binds to the ATP-binding site of the epidermal growth factor receptor (EGFR)-TK, which inhibits receptor tyrosine kinases (TKs) by inhibition of an intercellular domain. Usual side effects includes diarrhea and rash which sometimes requires dose reduction. Erlotinib, as well as other small-molecule anticancer drugs, is approved at a fixed dose. Association between certain toxicities and treatment efficacy has been demonstrated such as skin rash that might be used as a surrogate marker for effect. Alternatives to fixed dosing have been explored such as Therapeutic Drug Monitoring, genotype or phenotype adjusted dosing or dose adjustment according to toxicity. In early development of erlotinib, dose limiting toxicities was observed at 200mg/day and a dose of 150 was adopted as the maximal tolerated dose used in all subsequent studies (Karb 2000). An important observation in the preclinical studies with erlotinib was the direct relationship between target inhibition and antitumor effects in the animal model, suggesting that sufficient doses to inhibit the target in tumor tissues would need to be administered for this agent to be effective in the clinic.

Erlotinib (TKI) is widely distributed throughout the body and is metabolized in the liver by cytochrome P450s, primarily by CYP3A4 and CYP1A1, but CYP3A5 also has a minor role in erlotinib metabolism. In addition to its role as an ATP binding cassette transporter inhibitor, it is involved in inhibition of CYP2C8 and UGT1A1´s (a gene located on chromosome 2) activity. Hence, erlotinib may inhibit the metabolism of co-administered drugs that are substrates of CYP2C8 and UGT1A1 [McDonagh 2011].

The following points to several reasons for a large inter-individual variation in PK of erlotinib:

- Absorption and bioavailability is increased by food. It is advised that patient should take the drug without food, but variations in stomach emptying will have an influence on residual food and thus on absorption.

- Metabolism by the cytochrome P subtypes CYP3A4, CYP1A2 and CYP1A1, the activity of which are determined to various extent by heritage

- Interaction with other drugs metabolized with the above enzymes
- Presence of biologically active metabolites which kinetics and potency may vary among patients
- Long elimination half-life and low clearance which is a particular problem in patients with impaired liver function and high age.

- A large variation in the volume of distribution of erlotinib.

Erlotinib losses its pharmacological activity during phase I oxidative or reductive reactions [Klümpen 2011, Ling 2006]. Phase I products or parent drugs are conjugated through Phase II reactions that usually form inactive polar products readily available for renal and biliary elimination [Klümpen 2011].

The dosage suggestion is 150 mg erlotinib daily for adult patients according to the product label (medicin.dk); the drug should be taken at least 1 hour before or 2 hours after food intake. It has a bioavailability of 60%, which increases by food intake. After 7-8 days the steady state will be achieved. The main metabolites are biologically active and are eliminated via feces. The plasma half life is about 36 hours. Erlotinib is metabolized by CYP3A4, CYP1A2 and CYP1A1 [Ling 2006]. Concomitant use of CYP inducer or inhibitors may change plasma concentration of erlotinib. For example ketoconazole increases AUC of erlotinib by 85% and tobacco smoking conversely leads to increased elimination of erlotinib, probably due to induction of CYP1A2 [Ling 2006].

We propose that a fixed dose of erlotinib, due to the ADME factors, leads to a variable drug exposure in patients. As binding in the ATP pocket is reversible and happens in competition with ATP this would in turn lead to variations in effect and side-effects. It is possible that TKI's should be dosed on an individual level. The aim of this study is to assess the magnitude of the inter-individual plasma concentration of erlotinib as the first step in this process.

3. Method and material

The study is carried out in patients with NSCLC, who are treated with erlotinib, regardless of their previous treatment.

Inclusion criteria:

A confirmed diagnosis of non-small cell lung cancer

In, or about to start erlotinib treatment regardsless of stage

Age 18 years or older

Exclusion criteria:

Female patients should not be pregnant or lactating

Three sub-studies are defined as:

1. **Cross sectional**: a single sample taken after a minimum of 7 days of erlotinib treatment (to determine the person-person variability)
2. **Longitudinal**: several samples for each patient a minimum of 4 weeks apart (to determine variations within the individual)
3. **Initial:** Several samples taken with short interval at time of start of erlotinib treatment (to investigate ADME factors).

The same individuals can be part of all three sub-studies. There is no fixed interval between ingestion of erlotinib and blood sample for the cross sectional and the longitudinal study. For the longitudinal study a minimum of 4 weeks between samples are required. For the study of initial therapy, samples will be drawn at hours: 0,1,2,24,48,120.

A number of patient characteristics will be recorded in the patient´s CRF such as height, weight, smoking history, medication, and results of standard blood chemistry.

3.1 Sample taking

3.1.1 Blood sampling

A single blood sample (21ml) will be drawn from participating patients in sub study 1 and 2. The plasma will be separated and samples stored at -20C for later analysis. For sub study 1 and 2, blood samples can be drawn at any time after ingestion of erlotinib but an accurate time of ingestion and sample will be recorded. For sub study 3 each blood sample are 7 ml and must be taken at the time specified.

3.1.2 Sample treatment and storage conditions

The samples are centrifuged and labeled, plasma is separated and stored at -20 ^{0}C until analyzing by HPLC with UV detection [Zhang 2005].Studies have shown stability for erlotinib and the main metabolite OSI-420 in plasma stored under these conditions in excess of 1 year [Hamilton 2006]. Naïve plasma from untreated subjects is used for preparation of calibration standards and quality control samples. The quality control samples are used for method validation. A separate sample is send to the hospital Clinical Chemistry Department or to a commercial laboratory for analysis of CyP 450 subtypes.

3.1.3 Analysis method for determination of erlotinib concentration and its major metabolites

The content of the study entails planning and authority applications of the clinical trial, development and validation of analytical method of erlotinib and metabolites based on HPLC-UV, and integration of results to suggest an optimal dosing of erlotinib.
Reverse phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection is used to simultaneously determining plasma concentrations of erlotinib (OSI-774) and its major metabolite (OSI-420) [Zang 2005].

Chromatographic conditions:

The system consists of LC-10ADvp liquid chromatography system equipped with SPD-10 Avp UV detector. The analyses will be separated on a Water Symmetry C18 analytical column and a mobile phase consisting of -0.05 M acetonitrilenand 42:58 v/v potassium phosphate buffer. pH is adjusted to 4.8 with 0.2% triethylamine, and a flow rate at 1.0 ml/min choses. A detector wavelength of 345 nm is chosen.

Standard and stock solutions:

Stock solutions for erlotinib (OSI-774) and its metabolite (OSI-420) are prepared separately in duplicate by dissolving 2.0 mg of each drug in 10 ml pure methanol. A concentration of 200µg/ml is obtained, and then is stored at – 20^{0}C. The difference from the mean peak area in each of the duplicate stock solutions should be within 15%

Another stock solution (**Internal standard**) of OSI-597 is prepared by dissolving 2.0 mg drug in 10 ml pure acetonitrile, it should end with a concentration of 200µg/ml which is stored at – 20^{0}C. The internal standard is further diluted with acetonitrile to a final concentration of 10 µg/ml.

The stock solutions are further diluted each day with blank human plasma in order to prepare **calibration standards** of the following concentrations

Concentration ranges of calibration standards in ng/mL							
OSI-774 Erlotinib	12.5	100	500	1000	2000	3000	4000
OSI-420 Erlotinib metabolite	5	25	100	200	300	400	500

Table 3: Calibration standard of erlotinib and its major metabolite OSI-420.

A linear regression method/graph is used as calibration curve.

Quality control (QC) samples of OSI-774 and OSI-420 were prepared by mixing appropriate amounts of stock solutions and blank human plasma to obtain:

Quality Control samples (QC) ng/mL			
OSI-774	50	500	4000
OSI-420	25	100	500

Table 4: Quality Control samples used for method validation

The prepared QC samples are stored in batch at -20^{0}C for the duration of a validation procedure.

Sample preparation:

1) Plasma samples were thawed at room temperature.
2) 250µl plasma is transferred to a 100mm x 14mm polypropylene tube.
3) 25µl of the internal standard (IS) is added, mixed for 30 s on a vortex mixer.
4) 2.5 ml of methyl t-butyl ester is added, the tubes are capped and shaken on a shaker during 10 min.
5) The samples are then centrifuged at 3000xg for 10 min at ambient temperature. Transfer the supernatants to a clean glass tube.0.6 ml 5% HCL is added to the collected supernatants.
6) The mixtures are shaken during 30 min and then centrifuged at 3000xg for 10 minutes at ambient temperature. The organic layers are discarded.
7) 0.6 ml of 1N NaOH and 2.5 ml of methyl *t*-butyl ester were added to the remaining aqueous layer.
8) Again the samples are shaken for 30 minutes, afterwards centrifuged at 3000xg for 10 minutes under ambient temperature.
9) The supernatants are transferred to 1.5 ml polypropylene tubes and evaporated to dryness by using the Savant Universal vacuum system.
10) The residues are reconstituted with 0.25 ml of the mobile phase, mixed for 60 s on a vortex-mixer and centrifuged at 4000×*g* for 30 min at ambient temperature.
11) A volume of 100µl was transferred to a 250µl polypropylene auto-sampler vial.
12) 50 µl is injected onto the HPLC system for quantitative analysis.

Method Validation:

In order to find out the accuracy or percentage deviation of the assay, method validation are performed, a calibration curve is processed and the QC samples at low medium and high concentrations are run in quadruplicate.

Statistical methods:

The plasma concentration of erlotinib will be measured by the methods described above. The results will be depicted in graphs. The Standard deviation (SD) will be found. Further analysis is made with population based methodology. A population non-linear mixed effects analysis will be performed in which data from all the included patients are modeled simultaneously. The model building process is performed in a stepwise fashion. Co-variates (i.e. patient characteristics) are entered in the model by forward inclusion and backward deletion. The procedure is to include all co-variates one by one into the basic model and retain the model with the co-variate-parameter relation causing the largest significant improvement in the objective function value, OFV. This model serves as the new basic model and the stepwise inclusion procedure is repeated until no significant improvement is seen and this model then constitutes the full model. In the backward deletion the covariates in the full model is eliminated one by one until no more covariates can be eliminated from the full model without causing a significantly detrimental effect to the model fit. The statistical significance level will be set to 5% in the forward inclusion and to 0.1% for the backward deletion. The computer program, NONMEM®, will be used in the study.

4. Side effects, risks and benefits for patients that participates this project

The only study specific procedure for participating patients is a blood test which is drawn when the patients are seen in the out-patient clinic. A blood test may in some cases result in a small hematoma, but serious side effects is not seen. There will be no immediate benefit for participants.

Gaining more knowledge on metabolism and patient-patient variability may lead to further studies which may result in more effective and less harmful treatment of future patients.

Number of samples will be: 1 if the individuals accepts participation in the Cross sectional study, 2-3 of in Longitudinal, and 6 if in Initial study. Only patients who are about to commence in erlotinib treatment can be enrolled in the Initial study.

5. Permits and monitoring

The study will be conducted in accordance with the Helsinki declaration and GCP guidelines. The study will be initiated only after approval from the Ethic Committee and the National Board of Health/Danish Medicines Agency. The latter will have full access to source data for site inspection. The study will be monitored by a person appointed by the lead investigator. It is planned that the head of the Clinical Research Unit at Herlev Hospital will be appointed as study monitor. Plan for monitoring includes full monitoring of source data for the first 5 patients and then every 10.th patient. All signed informed consent

forms will be kept at the Research Unit. The database will be approved by the Danish Data Protection Agency

6. Economic conditions

The study is initiated by the investigators, MD Anders Mellemgaard, from Herlev hospital; and professor PhD Per Hartvig Honoré, at Copenhagen University, School of pharmaceutical sciences. The study is performed as a thesis by Khanda Amin.

None of the member of the research group has any financial interests in the project outcome. There will be no pay or other compensation for study investigators. None of the participating patients will get any sorts of remuneration.

7. Recruitment of participants

Potential participants are selected at the Oncology clinic at Herlev Hospital. Participation is voluntary and all other aspects of patient treatment will not be affected. The patient interviews will be made by the Chief Physician, Anders Mellemgaard, or another physician chosen by him. Current guidelines from the Science Ethics Committee will be followed.
The participants are encouraged to discuss their participation with friends or family. The conversation with the doctor will take place in a room at the hospital's department R. The patient is given both written information leaflet and a verbal description of the study. It is highlighted that participation is voluntary and that any participant may withdraw their consent at any time.

An eligible subject will be informed as part of the consultation which always takes place in a separate room. We encourage the presence of friends and family and the participant will be allowed amble time before they make their decision. Usually this means that the subject takes the information leaflet home and returns at some later time for the blood test should they decide to participate.

8. Information availability for participating patients

The patients will be informed about the experimental outcomes, if they have specified this in their consent form.

9. Publication of the results

The experiment results (regardless of what they may be) will be included in Khanda Amins thesis, which is assessed by the university guidelines. Results will further be sought published in an international peer reviewed journal.

10. The research ethic statement

For the individual who chooses to participate there is no immediate positive effect. For the general community however, this study will yield important information which may benefit lung cancer patients in the future. The risk for the participants is limited to the small hematoma that sometimes develops after a blood sample is drawn. We feel that this small risk for a un significant side effect is justifiable given the possible importance of the study.

The responsible physician will ensure that the experiment proceeds in accordance with the latest revision of the Helsinki Declaration and laws.

This study will be approved by the Science Ethics Committee (SEC) and the Data Inspectorate (DI). All the patients will be informed about the purpose of the study, both verbally and in writing.

Participation in the study is voluntary. At any time it is allowed for the patients to change their mind and withdraw their consent.

Participation don´t affect the patient's treatment or their relationship to the hospital department people. The consent will be stored in a folder, locked in an office, with limited access, located in clinical research unit, at oncology department R at Herlev Hospital.
In addition of that the patients will receive a copy of the consent while another copy will be putted in their record.

The science ethics committee must approve the application for the study, prior to start the experiment part of the study. There will not be further analysis or supplemental data collection without an additional permission from the RES.

The current participating patients will not get any benefits of their participation in the experiment of this project, they will just continue following their treatment plan. It will be beneficial to future patients to elucidate and clarify a possible problem.

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Annex 12.4: Case record form (CRF) containing clinical information

CRF i studiet ” Variation i plasmakoncentrationen hos patienter med ikke små cellet lungekræft ved behandling med fast dosis af erlotinib.

CPR nummer:_____ Initialer

Vægt:_____ kg Højde:_____ ___cm

BMI: _____

Dosering af erlotinib: _____mg/dag

Tid for sidste administration af erlotinib: Dato::_____Klokken

Tid for blodprøve af erlotinib: Dato::_____Klokken

Centrifugeret og mærket: Dato_____ Signatur:_____

Tidligere Cancer behandling:

Præparat: _____ Dosis: _____ mg Cyklus antal _____

Præparat: _____ Dosis: _____ mg Cyklus antal: _____

Præparat: _____ Dosis: _____ mg Cyklus antal: _____

Præparat: _____Dosis: _____ mg Cyklus antal.: _____

_____

Co-morbiditet:

_____

_____

Behandling med lægemidler for co-morbiditet:

Præparat: _____ Dosis: ___ mg Indikation:_____

Præparat: _____ Dosis: ___ mg Indikation:_____

Præparat: _____ Dosis: ___ mg Indikation:_____

Præparat: _____ Dosis: ___mg Indikation:_____

Præparat: _____ Dosis: ___mg Indikation:_____

Præparat: _____ Dosis: ___mg Indikation:_____

CRF over patientens kliniske data	
CPR nummer/ Patient nummer	
Initialer	
Erlotinib dosis	
Højde	
Alder	
Vægt	
Køn	
Ryger/ikke ryger	
Histologisk kræft form	
Co-morbiditet	
Brug af anden medicin	
Biokemi	

Annex 12.5: Note to manufacturer

Roche a/s

Industriholmen 59

2650 Hvidovre

Til den danske repræsentant for/fremstiller af erlotinib.

I henhold til lægemiddellovens vil vi hermed orientere om anmeldelsen af et klinisk forsøg til Lægemiddelstyrelsen, hvori erlotinib indgår

Forsøget har titlen: ” Variation i plasmakoncentration hos patienter med ikke små cellet lungekræft ved behandling med fast dosis af erlotinib”.
Forsøget har til formål at belyse sammenhænge mellem farmakokinetiske parametre, farmakodynamiske parametre og øvrige patientparametre under behandling med erlotinib.
Studiet vedrører NSCLC patienter som indtager 150 mg erlotinib dagligt.
Patienternes behandling er ikke en del af forsøget.

Med venlig hilsen

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Annex 6: Adverse event and monitoring:

Forsøgspersoner følges mht bivirkninger i 4 uger idet bivirkninger forventes at indtræde indenfor 1-2 døgn efter punktur.

Ved alvorlige bivirkninger (SAE) forstås en alvorlig hændelse eller bivirkning, som uanset dosis resulterer i død, er livstruende, medfører hospitalsindlæggelse eller forlængelse af hospitalsophold, resulterer i betydelig eller vedvarende invaliditet eller uarbejdsdygtighed eller fører til en medfødt anomali eller misdannelse.

SAE`er vil bliver skrevet på en liste og rapporteret en gang årligt til lægemiddelstyrelsen og videnskabsetisk komite sammen med en rapport om forsøgspersonernes sikkerhed.

SUSARs vil blive anmeldt til Lægemiddelstyrelsen indenfor 7 dage for alvorlige, 15 dage for ikke alvorlige. Produktresume udgør referencedokument i forhold til forventelige bivirkninger.

Elektronisk blanket vil blive anvendt:
https://blanket.laegemiddelstyrelsen.dk/Forms/ESUSARForm/ReportDetails/?languageid=4

Endeligt kan det oplyses at projektet vil blive monitoreret af repræsentant for Kliniks Forskningsenhed ved onkologisk afdeling og at der er udarbejdet en monitoreringsplan.